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F. H. Hesselink Van Suchtelen

F. H. Hesselink Van Suchtelen
1884–1937

On June 23, the day of his fifty-third birthday, Dr. F. H. Hesselink Van Suchtelen was killed in an automobile accident, which occurred only a few miles from Wageningen, Holland, the place of his birth. The name of Van Suchtelen is associated with few publications, but these occupy a rather unique place in the field of soil microbiology.

Born in Groenlo, Holland, in 1884, the son of the Burgomaster, he was graduated in 1904 from the Wageningen Agricultural High School. He worked for the next 6 years at Göttingen University under the leadership of Prof. Alfred Koch. The degree of doctor of philosophy (magna cum laude) was granted to him in 1910. After a year in Berlin and Leipzig, he came to the United States to become associated with the department of agricultural microbiology at Michigan State College and later at Massachusetts Agricultural College. In 1917, he returned to Europe, where he spent 13 years in München, as an independent investigator, utilizing the laboratory facilities of the Technological Institute. The year 1930–31 was spent at the laboratory of soil microbiology at Rutgers University. Again he returned to Germany, this time to the University of Giessen, where he devoted the following 4 years largely to library work in his selected field. He retired to private life in 1935.

The major contribution of Doctor Van Suchtelen to soil science was a study of the energetics of the soil and the rôle of microörganisms in energy transformations. The subject of his dissertation at Göttingen was the determination of the carbon dioxide evolution from soil as a measure of the aerobic activities of the microscopic population of the soil. He demonstrated that this process is a far more sensitive index of soil microbiological processes than is the enumeration of the bacteria developing on the plate. If one recalls that the solution culture method was much in vogue at that time and that the study of nitrogen transformation was considered to be all-important for measuring soil biological processes, the introduction of a new procedure based upon the study of the element carbon was more than welcome. He recognized the full significance of the carbon dioxide liberation in the decomposition of organic matter in soil and the rôle of microörganisms in this process. Although the evolution of carbon dioxide from soil was first studied systematically by Boussingault and Lewy in 1853, most of the subsequent workers limited themselves to a study of the carbon dioxide concentration in the soil air. Although Wollny, followed by a host of others, definitely established the significance of carbon dioxide evolution as a measure of soil microbiological processes, it was Van Suchtelen who demonstrated that carbon dioxide evolu-

tion from soil, with and without the addition of organic substances, can be used as a measure of soil microbiological processes and, therefore, of soil fertility.

This investigation was followed by a study of the soil solution and the soil environment for bacterial activities. From 1923–1931, he published a series of papers on the evolution of heat from soil. In these investigations he attempted to interpret soil microbiological processes from the standpoint of energetics. By making heat of combustion measurements of the soil organic matter and of added substances, he calculated the amount of energy available for the activities of the soil microörganisms. The evolution of heat, as a result of these activities, was found to depend on the fertility of the soil. A maximum of 4 per cent of the soil humus could be decomposed in the soil in one year. Anaerobic transformations were found to yield products which have a higher combustion value (per gram) than that of the products of aerobic decomposition processes.

During the final years of his active life, Doctor Van Suchtelen wrote several papers on the relation between chemical structure of organic substances and their decomposition by microörganisms.

By nature very retiring, Doctor Van Suchtelen did his best work when he was alone; he was the true independent investigator. His death creates a vacancy in a field which as yet has not attracted the attention that it fully deserves.

S. A. WAKSMAN.

FERTILITY VALUE OF CULTIVATED LAND AS INFLUENCED BY CROP-RESIDUE AND SEASON

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The influence of various factors, both climatic and edaphic, upon the composition of the soil, on the one hand, and the growth and yield of the following crop, on the other, has been studied from one point of view or another by an unusually large number of workers. As a result of careful experimentation with respect to season, nitrogen content of soil, and soil fertility, Burd and Martin (3), King and Whitson (6), Leather (7), Batham and Nigam (2), Fraps (4), and Greaves and Carter (5) among others came to the general conclusion that the growth and the yield of a crop are mainly associated with the ability of the soil to supply an adequate quantity of nutrients, that the nitrogen content of the soil varies with the season and with the nature of the plant grown, and that the higher the concentration of the nutrients in the liquid phase of the soil, the better is the fertility value. Although such investigations throw considerable light on the nature of inorganic nutrients of the soil, on the behavior of these nutrients from season to season and from plant to plant, as well as on their relation to yield of crops, very little is yet known about the nature, amount, and significance of the residual organic matter left after the harvest of a crop and their influence on the microorganic transformations in the soil.

In this paper efforts have been concentrated on a systematic study of cultivated land with special reference to the residual effect of leguminous and non-leguminous crops on the content of nitrogen, of moisture, and of organic matter (loss on ignition) of the soil. Seasonal fluctuations observed throughout the period of experimentation have been specially discussed in relation to variations in the environmental complex. The results of a separate experiment, on the green-manuring efficiency of certain leguminous crops, in progress,[1] the details of which will be given elsewhere, have also been briefly summarized, and the use of these crops in green-manuring is advocated.

PLAN OF EXPERIMENTATION

The investigations were conducted on the Experimental Farm of the Institute of Agricultural Research at Benares (table 1), where the soil lying within

[1] Singh, B. N., Kapoor, G. P., and Singh, M. P. Green-manuring efficiency of certain leguminous crops. Unpublished.

the alluvial belt of Ganges River is essentially sedimentary in nature. A field with uniform previous history was selected and divided into eight $\frac{1}{4}$-acre blocks. Records of the farm show that for the last 3 years the entire area under experimentation was sown with *Crotalaria juncea* in Kharif (rainy season) and with *Triticum vulgare* in Rabi (winter season) as detailed in table 2.

Crop plants belonging to both leguminous and nonleguminous orders were used on the blocks for comparative studies. *Cicer arietinum*, *Pisum sativum*, *Cajanus indicus*, and *Arachis hypogaea* represented the leguminous plants; and *Triticum vulgare*, *Saccharum officinarum*, *Gossypium herbaceum*, and

TABLE 1

Weather record at Benares from April 1934 to October 1934

MONTHS	TEMPERATURE		RAINFALL	RAINY DAYS	RELATIVE HUMIDITY	HOURS OF SUNSHINE
	Maximum	Minimum				
	°F.	°F.	inches		per cent	
April	103.8	72.1	0.1	1	28	381–56
May	107.9	79.7	0.0	..	30	412–20
June	103.0	83.2	3.6	3	62	406–20
July	91.8	79.7	14.8	13	82	415–40
August	90.6	79.1	12.6	13	84	399–22
September	86.6	75.4	1.2	2	50	365–46
October	80.5	72.8	....	..	35	354–40

TABLE 2

The history of the field for the last 3 years

YEAR	SEASON	CROP GROWN	NATURE	MANURE APPLIED
1931	Kharif	*Crotalaria juncea*	Leguminous	None
	Rabi	*Triticum vulgare*	Nonleguminous	Green-manure
1932	Kharif	*Crotalaria juncea*	Leguminous	None
	Rabi	*Triticum vulgare*	Nonleguminous	Green-manure
1933	Kharif	*Crotalaria juncea*	Leguminous	None
	Rabi	*Triticum vulgare*	Nonleguminous	Green-manure

Linum usitatissimum represented the nonleguminous plants. Of the four crops in each order, two are summer-sown and two are winter-sown. The crop plants were distributed in blocks at random. After the harvest of these crops the residual effect was studied. With the onset of rains each of these $\frac{1}{4}$-acre blocks was further divided into three plots, of which one was sown with a leguminous crop, *Crotalaria juncea*; another was sown with a nonleguminous crop, *Zea mays*; and the third was left fallow as control. The available nitrogen was estimated at intervals of 15 days throughout the period of experimentation in all the 24 plots. Finally the yields of all the crops grown therein were recorded.

EXPERIMENTAL TECHNIC

After the removal of all surface vegetable matter, a large number of soil samples were taken according to Official and Tentative Methods (1) from each of the plots at different depths. Samples from the same plot were mixed so as to give a composite mixture, representative of the entire area. All these samples were air-dried in the laboratory and were passed through a 1-mm. sieve. Each sifted sample was well mixed and preserved in suitable containers for analytical purposes.

The available nitrogen in the soil was estimated by the Devarda alloy method (1), and the moisture content was found by heating the sample to a constant weight in a steam oven regulated at 100°C. Total organic matter was determined by the loss in weight on ignition (1) by repeatedly heating the sample at low red heat in a platinum dish and desiccating it to a constant weight.

EXPERIMENTAL RESULTS

Moisture content

The mean moisture content of the soil ranges from 6.116 to 29.39 per cent (table 3). It varies independently of the type of the crop-residue and is markedly influenced by the season. With a fairly high value in spring, it reaches a minimum during summer when the intense heat of the sun depletes the soil moisture much below the normal. In the rainy season its value once again increases, reaching a maximum in July. The surface layer of the soil, in general, has a much lower water content than the deeper layers, where the fluctuations from season to season are not so well marked as in the upper layer.

Loss on ignition

The mean loss on ignition in all the plots ranges from 1.513 per cent, in the *Linum* plot, to 4.706 per cent, in the *Cajanus* plot (table 4). The loss in different plots varies greatly according to depth. Deep-root feeders exhibit the maximum loss in the deeper regions of the soil; shallow-rooted plants, in the topmost layers (table 4). Thus of the four leguminous crops, all except *Pisum* have a maximum loss at a depth of 12 inches. Among the nonleguminous plants, *Linum* appears to add to the organic content of the soil only in the top layers.

Available nitrogen

The amount of nitrate nitrogen is higher in the plots that were sown with leguminous crop plants than in the plots sown with nonleguminous plants. The symbiotic nitrogen fixers that are on the roots of the leguminous plant and that remain in the soil even after the harvest, seem to bring about the observed deviations in the nitrogen content.

The available nitrogen is found to vary also with the depth, which may

conveniently be ascribed to the fundamental effect of root development. With *Pisum* where roots are confined to the surface layers the nitrates are

TABLE 3

Moisture content at different depths of the soil as influenced by crop-residue and season

PREVIOUS CROP	DEPTH	SPRING		SUMMER				RAINS	
		April 5	April 20	May 5	May 20	June 5	June 20	July 5	July 20
	inches	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Pisum sativum	6	5.250	5.550	5.200	4.750	3.450	5.650	20.600	26.350
	12	8.400	8.450	7.650	6.800	6.400	6.730	19.500	26.500
	18	8.700	8.730	8.990	8.800	8.500	9.810	19.900	26.500
	Mean	7.450	7.576	7.280	6.683	6.116	7.396	20.000	26.450
Cajanus indicus	6	5.650	6.450	5.850	5.450	4.110	6.540	20.500	27.500
	12	8.500	9.500	8.000	6.250	4.950	8.810	21.900	27.900
	18	10.000	10.500	10.200	9.800	9.300	10.430	22.200	27.900
	Mean	8.050	8.816	8.016	7.166	6.120	8.593	21.533	27.760
Cicer arietinum	6	6.750	6.810	6.200	5.550	5.350	6.850	19.900	26.900
	12	8.800	8.900	7.400	6.900	6.550	8.970	21.800	27.800
	18	9.750	9.790	9.500	9.450	9.150	10.330	22.900	29.700
	Mean	8.433	8.500	7.700	7.300	7.016	8.716	21.566	28.100
Arachis hypogaea	6	6.500	7.450	6.450	5.850	7.250	7.230	22.500	26.810
	12	9.465	11.990	10.990	9.650	9.300	10.450	22.200	27.420
	18	10.200	12.950	12.250	11.650	11.450	11.580	22.800	27.400
	Mean	8.728	10.797	9.867	9.050	9.333	9.753	22.500	27.210
Triticum vulgare	6	5.360	6.550	5.890	4.995	4.700	6.110	20.200	28.400
	12	9.250	10.990	10.950	9.970	9.750	10.470	22.400	29.800
	18	9.550	12.800	11.900	11.400	10.900	10.800	23.500	29.970
	Mean	8.053	10.113	9.580	8.788	8.450	9.123	22.033	29.390
Saccharum officinarum	6	6.500	5.950	5.250	4.860	4.450	5.630	20.200	28.400
	12	9.460	9.450	7.870	7.100	6.900	8.310	22.400	28.100
	18	10.500	11.400	9.800	8.900	7.600	8.500	22.800	28.600
	Mean	8.820	8.933	7.640	6.935	6.317	7.480	21.800	28.367
Gossypium herbaceum	6	5.400	6.500	5.700	4.850	4.550	6.240	17.800	26.600
	12	7.750	8.900	7.100	7.170	7.070	10.760	17.760	26.710
	18	9.050	10.950	10.450	10.250	9.710	11.210	17.510	26.690
	Mean	7.400	8.783	8.083	7.423	7.110	9.403	17.690	26.667
Linum usitatissimum	6	5.640	6.300	5.890	4.765	4.450	6.450	21.490	28.120
	12	7.750	8.900	7.120	7.100	7.070	9.760	22.210	29.210
	18	9.500	11.750	9.150	9.080	9.250	11.140	21.800	29.620
	Mean	7.630	8.983	7.387	6.982	6.923	9.117	21.833	28.983

found to be highest in the upper 6 inches of the soil. *Cicer* and *Arachis* behave in a more or less similar manner. There is no characteristic feature of the

nonleguminous crops, where no relation seems to exist between the feeding level of the plant and the nitrogen value. *Gossypium*, for example, being a

TABLE 4

Loss on ignition at different depths of the soil as influenced by crop-residue and season

PREVIOUS CROP	DEPTH	SPRING		SUMMER				RAINS	
		April 5	April 20	May 5	May 20	June 5	June 20	July 5	July 20
	inches	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Pisum sativum	6	4.400	4.400	4.320	4.312	4.238	4.296	4.294	4.290
	12	3.860	3.720	3.860	3.540	3.526	3.521	3.500	3.480
	18	1.780	1.940	1.760	1.732	1.728	1.708	1.694	1.680
	Mean	3.346	3.353	3.313	3.194	3.146	3.175	3.163	3.150
Cajanus indicus	6	4.760	4.754	4.749	4.721	4.714	4.698	4.670	4.630
	12	4.880	4.870	4.841	4.822	4.729	4.760	4.740	4.720
	18	4.480	4.486	4.472	4.446	4.437	4.430	4.410	4.310
	Mean	4.706	4.703	4.687	4.663	4.648	4.622	4.606	4.553
Cicer arietinum	6	2.560	2.546	2.541	2.537	2.532	2.529	2.512	2.479
	12	2.760	2.741	2.721	2.702	2.687	2.674	2.642	2.627
	18	2.720	2.710	2.620	2.534	2.480	2.410	2.380	2.300
	Mean	2.680	2.665	2.627	2.591	2.566	2.537	2.511	2.472
Arachis hypogaea	6	2.980	2.870	2.810	2.674	2.759	2.746	2.721	2.710
	12	2.880	3.846	3.839	3.827	3.820	3.791	3.769	3.700
	18	2.020	1.970	1.940	1.928	1.910	1.894	1.881	1.876
	Mean	2.960	2.895	2.863	2.839	2.829	2.777	2.790	2.762
Triticum vulgare	6	4.200	4.098	4.040	3.862	3.846	3.821	3.791	3.760
	12	4.800	4.761	4.730	4.708	4.691	4.672	4.641	4.620
	18	2.640	2.631	2.629	2.621	2.617	2.594	2.582	2.570
	Mean	3.880	3.830	3.800	3.730	3.718	3.695	3.671	3.650
Saccharum officinarum	6	2.740	2.770	2.704	2.691	2.672	2.670	2.588	2.589
	12	3.960	3.940	3.926	3.910	3.887	3.876	3.860	3.820
	18	1.960	1.870	1.840	1.790	1.760	1.732	1.727	1.700
	Mean	2.886	2.860	2.823	2.797	2.773	2.759	2.725	2.701
Gossypium herbaceum	6	4.040	4.010	3.900	3.788	3.764	3.756	3.741	3.730
	12	4.080	4.060	4.021	3.981	3.970	3.940	3.911	3.890
	18	2.100	2.220	2.087	2.060	1.886	1.870	1.840	1.810
	Mean	3.406	2.096	3.334	3.276	3.210	3.188	3.164	3.143
Linum usitatissimum	6	2.080	2.100	1.980	1.941	1.900	1.880	1.840	1.800
	12	1.890	1.840	1.800	1.754	1.730	1.700	1.690	1.640
	18	1.220	1.200	1.180	1.170	1.141	1.119	1.109	1.100
	Mean	1.730	1.713	1.653	1.621	1.590	1.566	1.546	1.513

deep-root feeder, shows a greater amount of nitrogen in the upper 6 inches than in the medium depth of a foot, and in the 18-inch layer the content once

again increases. *Saccharum* and *Triticum* exhibit maximum quantities of nitrogen in the upper 12 inches of the soil.

TABLE 5

Available nitrogen at different depths of the soil as influenced by crop-residue and season

PREVIOUS CROP	DEPTH	SPRING		SUMMER				RAINS	
		April 5	April 20	May 5	May 15	June 5	June 15	July 5	July 15
	inches	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Pisum sativum	6	60	65	76	79	86	94	65	43
	12	43	51	59	65	66	68	66	30
	18	29	31	39	30	31	33	31	31
	Mean	44	49	55	58	61	65	54	38
Cajanus indicus	6	..	15	20	35	32	44	35	30
	12	33	25	45	45	66	61	55	46
	18	..	8	10	15	40	39	9	17
	Mean	11	16	25	32	47	48	33	31
Cicer arietinum	6	26	48	55	56	70	60	47	35
	12	49	54	68	64	81	92	86	61
	18	46	54	48	51	56	64	44	51
	Mean	37	56	57	57	69	72	59	49
Arachis hypogaea	6	69	75	78	79	90	89	91	78
	12	79	88	96	93	99	101	93	94
	18	59	68	68	66	75	74	66	54
	Mean	69	77	81	79	88	88	83	75
Triticum vulgare	6	..	25	38	48	52	56	22	18
	12	36	34	46	56	70	68	54	46
	18	..	20	30	20	48	48	44	30
	Mean	12	26	38	41	57	57	40	31
Saccharum officinarum	6	..	16	26	24	18	30	30	15
	12	9	24	30	18	44	54	48	22
	18	..	6	12	15	24	33	12	8
	Mean	3	15	23	19	29	39	30	15
Gossypium herbaceum	6	12	36	35	46	43	80	52	48
	12	4	15	28	22	40	48	43	28
	18	26	24	48	58	64	64	58	53
	Mean	14	25	37	47	49	64	51	43
Linum usitatissimum	6	12	32	29	46	52	62	54	48
	12	8	16	28	36	40	52	47	27
	18	4	12	15	23	31	33	28	18
	Mean	8	20	24	35	41	49	43	31

The available nitrogen in all the plots (table 5) is comparatively low in spring, increases in summer, and again decreases during the rains. The

TABLE 6

Yield, per plot, of succeeding crops as influenced by crop-residue and season

(Area sown, each plot, $\frac{1}{12}$ acre)

PLOT NUMBER	PREVIOUS CROP	*Crotalaria juncea*	*Zea mays*
		lbs.	*lbs.*
1	*Pisum sativum*	60½	30
2	*Cajanus indicus*	48	24
3	*Cicer arietinum*	70	30½
4	*Arachis hypogaea*	76½	36
5	*Triticum vulgare*	46½	26
6	*Saccharum officinarum*	44	24½
7	*Gossypium herbaceum*	46	25½
8	*Linum usitatissimum*	36½	18

TABLE 7

Available nitrogen at a depth of 9 inches as influenced by the standing crop

PREVIOUS CROP	STANDING CROP	AUGUST 1	AUGUST 15	SEPTEMBER 1	SEPTEMBER 15	OCTOBER 1	OCTOBER 15
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Pisum sativum	*Crotalaria juncea*	46	58	90	70	40	84
	Zea mays	20	10	41	8	40	26
	Fallow	41	42	43	44	49	43
Cajanus indicus	*Crotalaria juncea*	51	60	90	81	96	105
	Zea mays	38	42	50	40	80	44
	Fallow	49	55	63	62	72	39
Cicer arietinum	*Crotalaria juncea*	80	81	78	85	92	64
	Zea mays	54	42	31	26	19	31
	Fallow	55	78	85	91	96	86
Arachis hypogaea	*Crotalaria juncea*	111	115	109	117	113	105
	Zea mays	61	56	53	52	48	61
	Fallow	95	99	106	104	109	91
Triticum vulgare	*Crotalaria juncea*	78	81	92	95	89	83
	Zea mays	15	13	11	9	6	10
	Fallow	54	47	58	63	68	57
Saccharum officinarum	*Crotalaria juncea*	50	54	58	71	91	76
	Zea mays	16	14	12	12	9	14
	Fallow	39	35	38	44	45	55
Gossypium herbaceum	*Crotalaria juncea*	49	56	59	55	47	39
	Zea mays	20	15	14	12	8	15
	Fallow	35	29	31	40	51	39
Linum usitatissimum	*Crotalaria juncea*	69	71	82	88	88	83
	Zea mays	49	42	29	20	13	25
	Fallow	52	54	56	61	76	82

decrease in its value in different seasons of the year is more significant with the nonleguminous group than with the leguminous group.

It is thus noted that the availability of nitrogen in different seasons of the year depends to a large extent upon the crop growing in the field, together with certain other factors such as type of soil and temperature of soil and atmosphere. In the plots sown with nonleguminous crops the nitrogen content starts with a low value, which increases during the summer months, attaining the maximum on June 15. In certain plots sown with leguminous crops it starts with a relatively high value in the spring, increasing steadily during the summer. During the rains the nitrogen content declines markedly

TABLE 8

*Nitrogen content of the soil and yield of wheat per plot as influenced by the use of various leguminous crops**

AGE	CROPS				
weeks	*Crotalaria juncea*	*Sesbania acubata*	*Cyamopsis psoraloides*	*Phaseolus mungo*	*Phaseolus radiatus*
			Soil nitrogen		
4	0.172	0.180	0.200	0.154	0.168
5	0.194	0.187	0.204	0.160	0.170
6	0.196	0.200	0.210	0.174	0.171
7	0.210	0.202	0.224	0.177	0.182
8	0.224	0.216	0.230	0.180	0.177
9	0.240	0.219	0.229	0.196	0.200
10	0.243	0.221	0.241	0.200	0.210
			Yield of wheat, pounds		
Only roots left in the soil.....	19.5	17.0	20.0	17.5	17.5
Entire crop ploughed in.....	34.5	26.0	30.0	23.1	25.5

* The work is still in progress, and the data for several years calculated on a statistical basis will be presented as soon as they accumulate.

in all the plots, but when the atmosphere is clearer and precipitation does not take place for long intervals, the nitrogen content suddenly increases.

Yield of subsequent crops as influenced by crop-residue

The yields of both *Crotalaria juncea* and *Zea mays* undergo considerable variations depending upon the nature of the previously sown crop (table 6). Highest yields are obtained on the *Arachis*-sown plot. The results indicate, in general, that the plots sown with leguminous plants, which show greater nitrogen content, also produce high yields. That these differences are not due to any special treatment during the previous years is almost evident from the previous history of the experimental field (table 2).

The nitrogen content of the plots sown with *Crotalaria juncea* and *Zea mays*

was also determined, and it was found that the two plants deplete the soil nitrogen to different extents at different periods of observation. *Zea mays* exhausts the soil much more than does *Crotalaria juncea*. With *Crotalaria* the nitrogen content at successive stages, as well as toward the close of the observational period, is increased beyond the normal, as shown in table 7, possibly on account of the leguminous nature of the plant itself.

Green-manuring efficiency of certain leguminous crops

In a study of the green-manuring efficiency of leguminous crops, the crops were sown in $\frac{1}{40}$-acre plots, and the nitrogen content of the soil was studied beginning the fourth week after germination. Duplicate plots of each crop were sown side by side. At the end of the adolescent stage the plants in one plot were incorporated into the soil as described elsewhere (8), and those of the other were harvested, the roots being left in the soil.

From the data in table 8 it is obvious that a highly significant change is brought about in the nitrogen content of the soil by the cultivation of the leguminous crops. The variations in different cases are due probably to the efficiency of the nitrogen-fixing power of the individual species.

The yield of the succeeding crops also undergoes characteristic variations: the plots which were green-manured gave exceedingly high yields in comparison to the other set. *Crotalaria juncea* proved to be the best green-manure crop.

SUMMARY AND CONCLUSIONS

This paper deals with the effect of crop-residue and season on the amounts of moisture, available nitrogen, and organic matter (loss on ignition) in cultivated land.

The residual effect of leguminous and nonleguminous crop-residues on the available nitrogen of the soil varies very characteristically. In fields previously sown with leguminous crop the available nitrogen is relatively high as compared to that in fields sown with nonleguminous crops.

The amount of available nitrogen is highest in that stratum of the soil in which the plant feeds. Shallow-rooted legumes increase the quantity of surface nitrogen, and deep-rooted legumes exhibit the highest values in deeper layers. Fluctuations are very common in the top layers of the soil, the lowermost showing a steady and almost levelled nature. The greater the depth, the less is the available nitrogen.

The amount of available nitrogen in all cases is comparatively low in spring, increases in summer, and once again decreases in the rains, after which it again rises until the first of October. The decrease in its value with season is more significant with nonleguminous crops than with leguminous.

The loss on ignition of the soil varies from the early period of experimentation to the onset of rains. Deep-rooted feeders exhibit the maximum loss in the deeper regions of the soil; shallow-rooted ones, in the top layers.

The residual effect of leguminous and nonleguminous plants on the fertility

of land stresses the importance of the utilization of legumes in general in increasing the fertility of the land and in initiating a more profitable scheme wherein a due consideration is, of necessity, to be paid to the rotation of crops and the use of leguminous crops for green-manuring purposes.

Of the leguminous crops used in a study of manurial efficiency, *Crotalaria juncea* was found to contribute the most to the nitrogen content of the soil and to the yield of the succeeding crop.

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NITROGEN AND PHOSPHORUS CHANGES IN THE DECOMPOSITION OF RYE AND CLOVER AT DIFFERENT STAGES OF GROWTH[1]

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The supply of soil organic matter is derived principally from green manure plants and plant residues, which, upon decomposition, release plant food and indirectly increase the availability of plant nutrients in the mineral fraction of the soil. Since it has been suggested (12) that the value of organic matter in the soil is proportional to the rapidity with which the nitrogen is converted into nitrate, further studies seem desirable on the decomposition of some common green manure plants and the changes in the nitrogen and phosphorus during decomposition.

Evidence indicates that the rapidity with which the nitrogen is liberated from plant material depends upon the rapidity with which the material decomposes, which is, in turn, controlled by the age, chemical composition, and nitrogen content of the particular organic material. Hutchinson and Milligan (7) used nitrate accumulation in the soil as a measure of decay and found that the percentage of nitrification decreased markedly with the age of the green plant material added. Wright (24) found that the nitrogen of green manure undergoes vigorous nitrification. When resistant material was added, it reduced the rate of nitrification. Maynard (10) studied the rate of decomposition of sweet clover green manure and, using the accumulation of nitrate in the soil as a measure, found that the rate of decay decreased as the maturity of the plant tissue is approached. Merkle (11), using the rate of humus formation and evolution of carbon dioxide as a measure of the degree of decay, found that the greater the succulency of the material studied and the greater its nitrogen content, the more rapidly does it decompose. Hill (6) studied the rate of decomposition of oats, rye, clover, and vetch at different stages of growth and observed that the younger are the plants, the narrower is the C/N ratio, and that decomposition was much more rapid with young plants than with older plants. According to Waksman and Tenney (20) the rapidity of liberation of nitrogen in an available form depends upon the nitrogen content of the plant and upon the rapidity of the decomposition of the plant con-

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stituents. The same authors (21) further demonstrated that the total nitrogen present in the plant is not the only factor which controls the rapidity of decomposition but that the chemical composition of the material is of significance.

It has been shown also that when mature plants with wide C/N ratios are added to the soil, not only is nitrification at a minimum but the nitrogen is utilized by the microbial cell for synthesis, thus leading to nitrogen starvation of higher plants. Rahn (13), Waksman (18), Starkey (16), Whiting (22), and Jensen (8) are among the investigators who found that organic materials with wide C/N ratios decompose slowly and that soil treated with such materials becomes deficient in available nitrogen.

Information on the fate of phosphorus during decomposition of organic matter is very meager. Available evidence, however, indicates that considerable mobilization of phosphorus occurs during the decomposition of organic matter by microorganisms. Duschechkin (3) claims that in addition to physicochemical absorption of phosphorus, a biological absorption occurs during the decomposition of organic matter, the latter increasing with an increase in the organic matter content of the soil. Egorov (4) compared the soluble phosphorus content of a soil treated with an antiseptic with that of an untreated soil. He found a reduction in the soluble phosphorus content only in the untreated soil, indicating a conversion of inorganic phosphorus to organic compounds during the process of organic matter decomposition. Stoklasa (15) reported that bacterial cultures growing on media supplied with various insoluble phosphates may assimilate as much as 25 per cent of the total phosphorus, provided that suitable carbohydrates are present. Schreiner (14) was led to believe that microorganisms elaborate nucleo-protein from other nitrogenous and phosphatic compounds, including inorganic phosphates naturally occurring in the soil. Whiting and Heck (23) suggested that during the decomposition of materials with high cellulose content in compost, phosphorus might be added to advantage; it becomes converted into organic complexes. Demolon and Barbier (2) reported that when organic matter is mineralized, phosphates may be reassimilated by microorganisms and synthesized into microbial cells in the presence of available energy. Tam and Magistad (17) found a decrease in soluble phosphorus during the decomposition of organic matter; the unavailable phosphorus was eventually released. They attributed the decrease in soluble phosphorus to the utilization of the element by microorganisms during the active period of organic matter decomposition.

The purpose of this problem was to study the decomposition of total organic matter and the various organic constituents of rye and clover plants of different growth stages and to determine the changes in the nitrogen and phosphorus during decomposition. The phase of the problem dealing with the decomposition of the various organic constituents will not be discussed here.

EXPERIMENTAL

The air-dry samples of plant material described elsewhere (9) were used in this investigation. These were young clover plants, medium clover plants,

mature clover plants, as well as rye plants of corresponding ages. Two-hundred-gram quantities of the materials were placed in glazed pots, numbered 1 to 6. A duplicate sample of the mature rye was placed in a separate pot (number 7) and treated with additional mineral nutrients: 5 gm. ammonium sulfate, 2 gm. di-potassium phosphate, and 5 gm. calcium carbonate. Water was added in amounts equivalent to 350 per cent of the dry organic matter, giving a final moisture content of 77 per cent. All pots were covered with glass dishes and incubated at room temperature (21 to 23°C.).

The composts were well mixed at frequent intervals, and the moisture content was adjusted if necessary. At intervals of 30 and 80 days, aliquot samples of the materials were removed for analysis; moisture determinations were made, and the total amount of the original material remaining was calculated on an air-dry basis. At each sampling, allowance was made for the samples previously removed from each pot, and the amount of material left was again calculated on the basis of the total material originally introduced into the pots. Nitrate and ammonia were determined directly on wet material; other analyses were made on air-dry samples of the compost. Ten-gram portions of the moist material were used for ammonia and nitrate determinations. Normal potassium chloride solution was used to extract the ammonia, which was distilled with heavy magnesium oxide; nitrate was reduced with Devarda's alloy, and the ammonia which was formed was distilled into standard acid and titrated. Total phosphorus was determined by the A. O. A. C. method (1); organic and inorganic phosphorus were determined by the method of Heck and Whiting (5); total nitrogen was determined by the Kjeldahl method. Waksman and Stevens' (19) method was used for the proximate chemical analysis.

DECOMPOSITION OF TOTAL PLANT MATERIAL

The first determination of the total material decomposed was made after incubation for 30 days. The residual material left in the composts was weighed, the moisture content was determined, and the results were calculated on the basis of the total residual material. The rate of decomposition of the various materials is illustrated in figure 1. The lowest amount of residual material was found in the composts of the young plants, and the greatest amount, in the composts containing the mature plants. There was a difference, however, between the clover and the rye plants of the same age, the difference increasing with the age of the plants. It is noteworthy that more than half (51.51 per cent) of the young clover plants had decomposed at the end of 30 days, whereas less than half (46 per cent) of the young rye plants decomposed during the same period of time. On the other hand, the medium-aged clover compost was reduced by 43.38 per cent, and the rye compost of the same age, by only 29.47 per cent in 30 days. The percentage decomposition of the mature rye showed even greater divergency from that of the mature clover. The former was reduced by only 15.17 per cent of the original material, whereas the latter was reduced by 38.39 per cent. The mature clover

plants decomposed more than two and one-half times as rapidly as did the rye plants of the same age. When the mature rye compost received additional mineral nutrients, however, it decomposed twice as rapidly as the same material without additional nutrients.

The second determination of the loss of total organic matter was made after incubation for 80 days. The relation between the plants at the end of the 30-

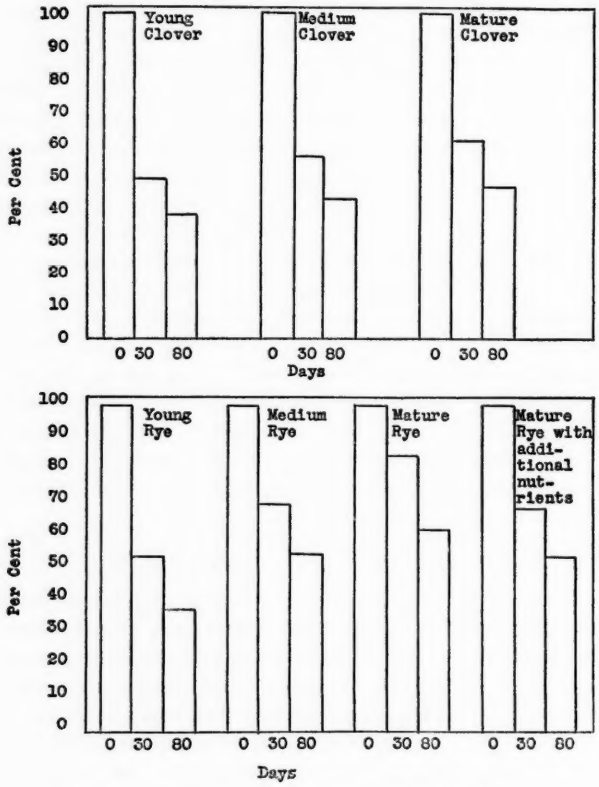

FIG. 1. DECOMPOSITION OF TOTAL PLANT MATERIAL

day period of decomposition was not the same as that at the second period. The young rye composts decomposed more rapidly than did the young clover composts during this period. At the end of 80 days, 62.35 per cent of the young rye compost and 61.30 per cent of the young clover compost had been decomposed; the order is the reverse of that during the first 30 days, when decomposition of the rye was considerably slower than that of the clover

plants. This fact gives further support to the results obtained previously (9), which showed that the young rye decomposed more rapidly than did the young clover, as measured by the evolution of carbon dioxide. The young clover compost contained a greater amount of water-soluble substances than did the young rye, which may account for a more rapid rate of decomposition during the first 30 days; but when the water-soluble constituents were exhausted, the rate of decomposition was retarded. On the other hand, the young rye contained more nitrogen and a larger amount of water-soluble nitrogen than did the clover plants of the same age; the nature of the nitrogen compounds and the complexity of some of the other constituents may be responsible for the limited destruction of the young rye plants during the initial 30-day period. As to the medium-aged composts, 56.60 per cent of the clover and 44.40 per cent of the rye had decomposed at the end of 80 days, indicating the same difference in the rate of decomposition as that shown at the end of 30 days. The mature clover compost lost 52.10 per cent of its total material, and the mature rye, 36 per cent, in the same period. This indicates a more rapid rate of decomposition of the mature rye during the latter period of incubation than during the earlier period.

The reason for the difference in the amounts of organic matter lost from the composts of plants of different stages of maturity can be explained from analysis of the plant constituents that remained in the compost. This disappearance of hemicelluloses, celluloses, fats, waxes, and water-soluble constituents accounts for the greater portion of the loss of total organic matter. The lignin and the crude protein, being more resistant to decomposition, remained in the compost. In the young plant composts, all of the organic constituents, except lignin and crude protein, decomposed more rapidly than did the total organic material. The latter complexes account for most of the residual organic matter remaining in the humus. Some mineralization of the protein and loss of lignin from the young plant compost occurred, however, after prolonged decomposition. On the other hand, analysis showed that the organic constituents, except the water-soluble substances, of the older plants resisted decomposition and that the former were present in the mature plant compost in much greater abundance than in the young plant compost. The cellulose and hemicellulose had decomposed to a limited extent; the lignin had either increased or remained unchanged; and there was an absolute increase in the crude protein. It has been shown (2) that the rate of decomposition of organic materials depends upon the amount of water-soluble substance present and upon the nitrogen content of the particular organic matter. The young plants, consisting of more than one third water-soluble constituents and containing a high nitrogen content, decomposed much more rapidly than did the mature plants with a small amount of water-soluble substances and low nitrogen content. This fact is further supported by the mature rye compost which received additional nitrogen; there was a much

greater loss in the total organic matter, and the organic constituents decomposed much more rapidly than did those of the same compost without additional nitrogen.

Transformation of nitrogen

Analysis of the nitrogen transformed in the various composts was made after decomposition for 30 days. The quantities of nitrate and ammonia liberated from the clover and the rye plants at different stages of maturity, during the process of composting, ran parallel to those liberated from the same materials in the soil (9). The data presented in table 1 show that in the young clover compost 3.29 mgm. of ammonia and 2.91 mgm. of nitrate were produced; the ammonia represented 7.92 per cent and the nitrate 7.02 per cent of the total nitrogen present in the compost. On the other hand, in the rye compost of the same age 4.4 mgm. of ammonia and only 0.7 mgm. of nitrate were produced during the same period; the ammonia liberated was 14.28 per cent of the total nitrogen, and the nitrate, 2.24 per cent. In the medium-aged plants, 4.1 mgm. of ammonia, equivalent to 15.74 per cent of the total nitrogen, and no nitrate were liberated from the rye compost; whereas in the clover compost 3.14 mgm. of ammonia and 0.32 mgm. of nitrate, equivalent to 10.61 per cent and 1.08 per cent, respectively, of the total nitrogen found in the compost, were liberated. Most of the nitrogen transformed in the mature plant composts evidently was consumed by the microorganisms during the process of decomposition of the materials, but to a greater extent in the rye compost than in the clover. The increase of the protein content in these composts supports this statement. In the mature clover compost, 1.4 mgm. of ammonia, or 5.5 per cent of the total nitrogen, was liberated; in the mature rye compost only 0.31 mgm. of ammonia nitrogen, representing 3.90 per cent of the total nitrogen was liberated. In the presence of a large amount of undecomposed organic matter, one could not expect an accumulation of nitrate, since the presence of ammonia has an inhibitory effect on the production of nitrate by microorganisms; it is not until the period of rapid decomposition is completed and virtually all the available organic compounds are used up that the nitrifying bacteria become active. This is observed in the compost where a large amount of organic matter remained undecomposed, whereas in the presence of considerable amount of ammonia, no nitrate accumulated. In the mature clover, medium rye, and mature rye composts a large portion of the organic matter was still undecomposed; therefore, no nitrate accumulated. When more than one half of the organic matter in the young clover had been destroyed, 2.91 mgm. of nitrate was found per gram of compost. On the other hand, where more than one half of the organic matter remained undecomposed, as in the young rye compost, only 0.7 mgm. of nitrate was formed. In the young rye plants, ammoniacal nitrogen was rapidly formed in soil as well as in compost, but the liberated ammonia was slowly nitrified. The relative amount of total nitrogen in the various materials showed a decided increase after the initial 30-day period of decomposition. The increase in the

total nitrogen is explained by the fact that some of the carbonaceous material had been destroyed and the nitrogen contained therein was assimilated by the microorganisms and synthesized into microbial protein. These results do not indicate fixation of nitrogen.

The nitrogen mineralized in most of the composts after decomposing for 80 days was considerably less than that mineralized during the first stage of decomposition. From the aforementioned mineralization of the protein in the young plant compost after 80 days, one naturally would anticipate the liberation of a greater amount of nitrogen. The results presented in table 1 show that 1.55 mgm. of ammonia and 1.36 mgm. of nitrate nitrogen were liberated from the young clover after 80 days' decomposition, compared with 3.29 mgm. of ammonia nitrogen and 2.91 mgm. of nitrate in 30 days. The young rye compost contained 1.5 mgm. of ammonia and 0.65 mgm. of nitrate

TABLE 1

Transformation of nitrogen during the decomposition of clover and rye

N expressed in milligrams per gram of material

	ORIGINAL MATERIAL	DECOMPOSITION 30 DAYS					DECOMPOSITION 80 DAYS				
	Total N	Total N	Ammonia N		Nitrate N		Total N	Ammonia N		Nitrate N	
	mgm.	*mgm.*	*mgm.*	*per cent of total N*	*mgm.*	*per cent of total N*	*mgm.*	*mgm.*	*per cent of total N*	*mgm.*	*per cent of total N*
Young clover	31.5	41.44	3.29	7.92	2.91	7.02	40.32	1.55	3.8	1.36	3.37
Medium clover	24.6	29.6	3.14	10.61	0.32	1.08	25.86	1.30	5.0	0.64	2.22
Mature clover	14.9	23.5	1.4	5.50	....	....	21.76	3.0	13.7	0.3	1.35
Young rye	36.5	38.9	4.4	14.28	0.7	2.24	43.12	1.50	3.7	0.65	1.5
Medium rye	17.8	25.4	4.1	15.74	....	....	27.44	4.8	13.10	....	....
Mature rye	6.8	7.8	0.31	3.96	....	....	10.08	0.2	1.9	....	....
Mature rye*	6.8	13.3	0.44	3.30	....	....	17.04	0.35	2.20	....	....

* With additional mineral nutrients.

per gram after 80 days, compared with 4.4 mgm. of ammonia and 0.7 mgm. of nitrate after 30 days. The amount of ammonia decreased in the medium clover compost and increased in the mature clover compost. The medium rye showed an increase in the amount of ammonia, but none of the ammonia was nitrified after a period of 80 days, whereas the ammonia in the mature rye compost decreased as compared with the amount liberated during the 30-day period. It is interesting to note that the total nitrogen content of the clover plants, after the 80-day period of decomposition, was reduced below the nitrogen content of the same plants after the 30-day period of decomposition, and the total nitrogen content of the rye plants increased above that present in the same plants after 30 days of decomposition. The decrease in the total nitrogen in the clover compost and the difference not accounted for as available nitrogen liberated in the compost indicate nitrogen lost during the de-

composition process. The increase in the total nitrogen of the rye plants indicates that the nitrogen was assimilated by microorganisms. Loss of nitrogen from the young clover and the young rye plants was evidenced by a marked odor of ammonia 65 to 70 days after decomposition started. The odor of ammonia escaping from the compost was so obvious that no qualitative test was necessary. The loss of ammonia probably accounts for the small amount of ammonia and nitrate present in the composts of the young plants. The decomposition of young materials rich in nitrogen involves many considerations of practical interest, since rapid decomposition may lead to volatilization of ammonia and consequent loss of nitrogen. The clover plants in all stages of maturity liberated nitrogen in an available form more rapidly than did the rye plants of corresponding ages.

Mobilization of phosphorus

The forms of phosphorus in the composts at various periods during decomposition are interesting when compared with the forms of phosphorus in the original materials. The data presented in table 2 show that an increase occurred not only in the total phosphorus content of the materials, but also in the organic phosphorus content; and that, moreover, a decrease in the inorganic phosphorus occurred as decomposition advanced. In the original plant materials, a larger proportion of the total phosphorus occurred in the inorganic form than in the organic form. The inorganic phosphorus content of the different plant materials ranged from 62.70 per cent to 70.03 per cent of the total phosphorus, and the organic phosphorus content of the various materials ranged from 29.97 per cent to 37.30 per cent of the total phosphorus. After decomposition had progressed for 30 days, there was a reversal in the distribution of the organic and inorganic phosphorus present. After decomposition for 30 days, the organic phosphorus ranged from 48.30 per cent to 64.77 per cent of the total phosphorus; and the percentage of inorganic phosphorus had decreased, ranging from 35.23 per cent to 51.70 per cent of the total phosphorus. The age of the plant as well as the nature of the plant influenced the conversion of inorganic phosphorus into the organic form during the process of decomposition. A greater portion of the inorganic phosphorus was converted into the organic form during the decomposition of the clover plants than of the rye plants; the amount also increased with the advance of maturity of both clover and rye plants. The results lead one to conclude that during the process of decomposition of organic materials and the mineralization of the inorganic compounds, the phosphorus is assimilated by the microorganisms and elaborated into lipids, nucleo-proteins and other organic phosphorus compounds; thus, the phosphorus becomes immobilized. Later, however, it is again made available upon the disintegration of the organic phosphorus compounds after death of the microbial cells. The mature rye compost which received additional mineral phosphate yielded evidence to bear out this hypothesis. In the compost of the mature material without

TABLE 2

Mobilization of phosphorus during the decomposition of legume and nonlegume plants

	ORIGINAL MATERIAL					DECOMPOSITION 30 DAYS					DECOMPOSITION 80 DAYS				
	Total phosphorus	Inorganic phosphorus		Organic phosphorus		Total phosphorus	Inorganic phosphorus		Organic phosphorus		Total phosphorus	Inorganic phosphorus		Organic phosphorus	
	Per cent of total material	Per cent of total material	Per cent of total P	Per cent of total material	Per cent of total P	Per cent of compost	Per cent of compost	Per cent of total P	Per cent of compost	Per cent of total P	Per cent of compost	Per cent of compost	Per cent of total P	Per cent of compost	Per cent of total P
Young clover	0.634	0.431	67.98	0.203	32.02	1.23	0.525	42.72	0.704	57.82	1.30	0.819	64.22	0.474	35.88
Medium clover	0.397	0.269	67.76	0.126	32.24	0.752	0.294	39.20	0.458	63.00	0.835	0.371	43.49	0.464	54.51
Mature clover	0.225	0.186	62.70	0.096	37.30	0.538	0.194	36.00	0.344	64.00	0.571	0.269	49.12	0.302	52.88
Young rye	0.641	0.430	67.25	0.211	32.75	1.18	0.62	51.70	0.560	48.30	1.40	0.916	64.00	0.484	36.00
Medium rye	0.458	0.350	70.03	0.088	29.97	0.66	0.262	40.0	0.398	60.0	0.565	0.313	55.40	0.252	44.60
Mature rye	0.229	0.161	63.27	0.066	36.63	0.32	0.141	41.18	0.182	58.82	0.343	0.188	54.80	0.155	45.20
Mature rye*	0.229	0.161	63.27	0.066	36.63	0.56	0.189	35.23	0.366	64.77	0.754	0.390	52.00	0.364	48.00

* With additional mineral nutrients.

additional phosphate, 58.82 per cent of the total phosphorus was present in the organic form and 41.18 per cent in the inorganic form; whereas in a similar compost with mineral phosphate, 64.77 per cent of the total phosphorus was present in the organic form and 35.23 per cent in the inorganic form.

The second analysis of the material was made after decomposition had progressed for 80 days. The data presented in table 2 indicate that mobilization of the phosphorus had taken place to a considerable extent. The range of total phosphorus in the organic form was from 35.88 to 54.51 per cent in the various materials after decomposing for a period of 80 days, as compared with a range of 29.97 to 37.30 per cent originally. It was clear that the organic and the inorganic forms of phosphorus were thus approaching the level of the original materials, as compared with the percentage of total phosphorus in the organic form after the initial period of decomposition. From the increase in the inorganic phosphorus during 80 days of decomposition, it is assumed that a large portion of the inorganic phosphorus which was converted into the organic form during the early period of decomposition became liberated from the microbial cells during the period of advanced decomposition of the organic material. The young plants which decomposed more rapidly showed less phosphorus in the organic form after 80 days; whereas most of the organic phosphorus persisted in the older plants. The greater portion of inorganic phosphorus found in the young plant compost after 80 days might be explained by the rapid decomposition of the organic material contained in the composts, a circumstance which resulted in a slowing down of cell synthesis or in death to the microbial cells, accompanied by the rapid liberation of phosphorus from the microbial cells. The process of fixation of phosphorus in the microbial cells and of the subsequent liberation is important from the point of view of providing available phosphorus for plant nutrition. If the phosphorus were liberated immediately in the soil, a large portion might become permanently fixed in forms which are unavailable to higher plants.

SUMMARY

Studies were made on the decomposition of clover and rye plants of different growth stages and on the changes in the nitrogen and phosphorus during decomposition.

The rapidity of the decomposition of the various plant materials was markedly influenced by the age of the plant: the younger the plant, the more rapid was the loss of total organic matter.

During the early period of decomposition the clover plants of all stages of maturity decomposed more rapidly than did the rye plants of the same age, but on further decomposition the young rye plants lost a greater amount of total organic matter than did the young clover.

The disappearance of hemicelluloses, celluloses, and water-soluble constituents accounts for the greater portion of the loss of the total organic matter.

In the young plant compost, the lignin and protein complexes account for

most of the residual organic matter, whereas hemicellulose, cellulose, and lignin account for most of the organic matter remaining in the composts of the mature plants.

Mineral nitrogen was liberated from the clover plants more rapidly than from the rye plants of the same age.

Ammonia was formed more rapidly from the young rye plants than from the young clover plants, but the ammonia was slowly nitrified.

The rapidity with which mineral nitrogen is liberated is influenced by the chemical composition of the plants. Young plants contain a relatively large amount of nitrogen and a relatively low percentage of organic complexes. They decompose rapidly, therefore, and a large amount of mineral nitrogen is produced.

Because of rapid decomposition, nitrogen may be lost through volatilization of ammonia.

During the process of decomposition of organic materials, inorganic phosphorus is converted into organic phosphorus through assimilation by microorganisms and is elaborated into organic cell substances.

Upon further decomposition of the organic materials, the phosphorus is again liberated into the inorganic state; this appears to be associated with the disintegration of the microbial cells.

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THE RELATION OF EXCHANGEABLE CATIONS TO THE "ACTIVE" ALUMINUM IN SOIL

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Efforts have been made by several workers to explain the problem of so-called "active" aluminum in soil. It has been ascertained that aluminum, like other toxic substances, in small quantities stimulates the growth of plants (6, 8, 9) and in larger quantities causes toxicity and sometimes destroys field crops and trees. Detriment to crop production has in many cases been explained as a consequence of the presence of "active" aluminum in soil (4). It has been shown, further, that acid fertilizers that lower the pH of soil release aluminum and thus decrease the crop yield (2).

Significant quantities of free Al_2O_3 are found in the degraded sandy soils of Palestine, and in the general problem of improving these soils the question of the origin of "active" aluminum has arisen (7).

CATIONS IN THE ABSORBING COMPLEX AND THEIR INFLUENCE ON SOIL PROPERTIES

Wiegner and others (5, 10, 12, 13), by measuring the viscosity and the ultramicroscopic coagulation of suspensions of clays saturated with various cations, have determined the relation between the positions of the cations in the Hofmeister series and the properties of clays saturated with these cations, with reference to water absorption and coagulation. As a general rule, it was found that with the increase in the atomic weight of the monovalent and the divalent cations fixed by the soils, the water-absorbing capacity decreases and the coagulating capacity increases. It has likewise been found that the penetrating force of a cation into the absorption complex is proportional to the equivalent weight of the penetrating cation (12). The amounts of water retained under equal pressures by clays saturated with different cations depend on the position of the cations in the Hofmeister series.

Aarnio (1) in his study of the influence of adsorbed ions on soil reaction ascertained that the pH of clay saturated with a monovalent or divalent cation decreases with the increase of the atomic weight of the cation and is lower for clay saturated with divalent cations than for clay saturated with monovalent cations. The physicochemical properties (electrokinetic potential, hydration, diameter of molecules) of such clays are determined by the reactions which

[1] The authors express their thanks to G. B. Baker, government analyst, for his kind advice and help.

these cations exhibit in simpler mineral compounds (13). It was natural, therefore, to suggest that the *absorbed cation may have a specific influence on the stability of the complex and on its capacity for slaking. The "active" Al in the soil is thus a function of the cation absorption of clays.*

DETERMINATION OF "ACTIVE" ALUMINUM IN SOILS SATURATED WITH DIFFERENT CATIONS

With a view to verifying the theory suggested, samples of two soils saturated with different cations were prepared. These soils comprised, first, red loamy

TABLE 1

"Active" Al in red loamy soil of Atharoth before and after treatment with salts

CATIONIC SOILS	"ACTIVE" Al_2O_3	pH
	p.p.m.	
Initial soil	95	6.8
H	98	5.6
Li	85	7.4
Na	35	7.2
K	24	6.8
Mg	90	7.3
Ca	25	7.4
Ba	25	7.4

TABLE 2

"Active" Al in heavy alluvial soil of the coastal plain (Yarkon River) before and after treatment with salts

CATIONIC SOILS	"ACTIVE" Al_2O_3	pH
	p.p.m.	
Initial soil	90	7.0
H	100	5.6
Li	90	7.5
Na	40	7.3
K	28	6.7
Mg	98	7.1
Ca	20	7.3
Ba	16	7.3

soil of Atharoth (near Jerusalem), which developed *in situ* on calcareous rocks of a low $CaCO_3$ content (0.5 per cent), and, second, heavy alluvial soil deposited by Yarkon River in the coastal plain.

These soils were treated with solutions of HCl, LiCl, NaCl, KCl, $MgCl_2$, $CaCl_2$, and $BaCl_2$ and were then washed with distilled water until chlorine-free, the corresponding H-, Li-, Na-, K-, Ca-, and Ba-soils thus being obtained. The amounts of "active" Al_2O_3 in these soils were determined by the Burgess method (3), the soil being leached with 0.5*N* acetic acid and the Al determined as $AlPO_4$. The results are recorded in tables 1 and 2.

From the data it is evident that *the influence of the Hofmeister series established for the atoms is not confined to the aforementioned soil properties but embraces also the stability of the complexes saturated with the corresponding cations*, the amount of "active" Al_2O_3 being regarded as the measure of stability. The "active" Al_2O_3 in soil may be regarded as a part of the "colloidal soil complex," to which it is bound with more or less strong ties. The conditions governing the presence of "active" Al_2O_3 in soil will therefore be the same as those governing the stability of the "complex." The stability of the "colloidal soil complex" is a function of the displacing power of cations, which according to Wiegner depends on the position of the cation in the Hofmeister series. Our experimental data also corroborate the observations made by Wiegner in his study of the "Eintauschkonstante" for various cations in ammonium permutite (table 3). The figures for the "active" Al_2O_3 in soil and those for the aforementioned "Konstante" are comparable except that they appear in reverse order, which means that a decrease in "active" Al_2O_3 is tantamount to an increase in stability of the "complex."

TABLE 3

The "Eintauschkonstante" in ammonium permutite for various cations according to Wiegner(11)

CATION	"KONSTANTE"
Li	15.210
Na	29.462
K	45.070
Mg	29.802
Ca	48.128
Ba	69.187

The amount of "active" Al_2O_3 in soils with the respective cations will depend on the position of the replaceable cations in the Hofmeister series. The high Al_2O_3 value found for the H-soil is in agreement with the many observations made upon its state as a strongly dispersed system disposed to disintegration.

The observations which were made on the presence of "active" Al in soil in connection with the application of acid and basic fertilizers may be easily explained on the basis of the relations existing between exchangeable cations and the stability of the absorption complex. The study of the saturation state of the soil-absorption complex is of importance inasmuch as *the phenomena of weathering in soils depend on the absorbed cation or cations.* The state of saturation, both qualitative and quantitative, influences the degree of disintegration of the "complex" and, as a result, affects both the mobilization of the plant nutritive elements and the appearance of toxic compounds like Al_2O_3 in the soil.

From the practical point of view, the data in this paper show that Ca and K salts can improve the soil by reducing the "active" Al_2O_3 to the lowest level, about 25 p.p.m., and that Mg and Li increase the Al_2O_3 soil-toxicity to 85–98 p.p.m.

SUMMARY

A close relation exists between the position and valence of an absorbed cation in the Hofmeister series and the amount of "active" Al_2O_3 in the soil, and this results in the changing stability of the absorption complex. The state of saturation with regard to cations influences the weathering phenomena and through them the appearance of toxic Al_2O_3.

The conditions established corroborate observations made by other scientists in connection with the application of acid and basic fertilizers.

K and Ca salts improve the soil by decreasing the content of "active" Al_2O_3, whereas Li and Mg salts increase the "active" Al toxicity of soil. The maximum "active" Al_2O_3 was found in H-soil.

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A METHOD FOR THE DETERMINATION OF THE ORGANIC PHOSPHORUS OF SOILS[1]

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It has long been recognized that significant proportions of the total soil phosphorus occur in organic combination. The plowing under of green manures, the addition of stable manures, the decay of plants, and the addition of organic fertilizers may contribute directly to this form of phosphorus in the soil; and microorganisms are continually synthesizing complex proteins which contain organic phosphorus. On the other hand, the quantity of organic phosphorus in the soil at any one time is the result of destructive forces as well as the aforementioned constructive ones. The activities of bacteria, fungi, and protozoa may also be counted among some of these destructive forces. Enzymes liberated by plants may hydrolyze the complexes containing phosphorus, and some acid hydrolysis may also occur. Schreiner (12) has shown, moreover, that plants are stimulated by the addition of nucleic acid to nutrient solutions and that the nucleic acid content of the solutions is lowered. This observation suggests the possibility that crop plants may utilize organic phosphorus either directly or after hydrolysis of the organic compound and thereby lower the organic phosphorus content of the soil. Other important factors which are still unknown may be influencing this dynamic equilibrium between organic and inorganic phosphorus in the soil. In view of these facts, a method whereby the total organic phosphorus of the soil could be estimated would be an aid in the study of this complex problem.

Despite the immense amount of work that has been done on soil organic matter, no one has offered a simple, easy method for the determination of this large fraction of the soil phosphorus. Stewart (14), using the method of Hopkins and Pettit (7), calculated the organic phosphorus from the carbon:phosphorus ratio by means of a factor derived from the nitrogen:carbon ratio and the organic nitrogen:phosphorus ratio. He assumed that there was no organic phosphorus in the subsoil. This type of reasoning is now known to be erroneous. Schmoeger (11) suggested hydrolyzing the soil under pressure at a temperature of 140 to 160°C., followed by extraction for 24 hours with

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cold 12 per cent hydrochloric acid. The difference between this value and the amount extracted from the untreated soil was taken as organic phosphorus. A similar method calls for ignition followed by extraction with cold hydrochloric acid as in the foregoing method, and the amount originally extracted is subtracted from the ignition value to give the amount of organic phosphorus. Such strong acid may dissolve some of the organic phosphorus from the untreated soil and thereby lower the values; a more general criticism is that ignition causes appreciable solution of iron and aluminum phosphates, as shown by Fraps (6). This latter criticism may apply to the method recently recommended by Odynsky (8), who advocates ignition at 600°C. for 1 hour followed by extraction with 2.0 *N* sulfuric acid. Shollenberger (13) confirmed the work of Fraps on ignition and published a modification of Potter and Benton's method (10) based on the solubility of the organic matter in dilute ammonia which seemed to give reliable values. This method is too long and complicated, however, to be used for routine laboratory work.

Since an accurate measure of the organic phosphorus would furnish a valuable tool in studying soil organic matter in general, and the phosphorus problem in particular, a method which readily lends itself to routine determinations has been devised in this laboratory. This method is based upon hydrogen peroxide oxidation of the soil organic matter. Hydrogen peroxide is known to oxidize about 90 per cent of the soil organic matter and to have no significant effect on the inorganic constituents. Phosphorus is extracted from the soil with 0.2 *N* sulfuric acid, and the difference between the phosphorus content of the extracts of the oxidized and the unoxidized soil is taken as a measure of the organic phosphorus. Peterson (9) was the first to use peroxide in the study of organic phosphorus. He found a large increase in the phosphorus extracted by 0.2 *N* nitric acid after oxidation with hydrogen peroxide. Unfortunately he does not state the purity of the peroxide used. Even high grades of hydrogen peroxide are likely to contain appreciable amounts of phosphorus because phosphoric acid is used as a stabilizer in many peroxide solutions. Phosphorus must be removed before the peroxide can be employed in this work. Doughty (5), studying phosphorus fixation, oxidized a peat with purified peroxide and found that this treatment reduced the fixation of added phosphate from 157 p.p.m. to 5 p.p.m. He attributed this reduction in fixing power to a partial saturation of the fixing material with the phosphorus liberated from the organic matter during oxidation.

Auten (3), using on Iowa soils the old method of Potter and Benton (10) as modified by Shollenberger, found that the organic phosphorus varied considerably in different soils and also within the same soil profile. In addition he postulated various compounds in which the organic phosphorus might occur (2). Aso (1) isolated a lecithin from a soil; other work indicates that soils may also contain organic phosphorus as phytin, nucleic acids, proteins, and their decomposition products. Before the isolation and the identification of individual organic phosphorus compounds are undertaken on a quantitative basis, however, the determination of the total organic phosphorus is necessary.

DESCRIPTION OF THE METHOD

Reagents

Sulfuric acid for extraction. Dilute concentrated sulfuric acid, approximately 35 *N*, to 17.5 times its original volume. Titrate a small sample with standard alkali and adjust to 2.0 *N*.

Ammonium molybdate-sulfuric acid solution. Dissolve 25 gm. of ammonium molybdate in 200 ml. of water heated to 60°C.; filter. Dilute 275 ml. concentrated sulfuric acid to 800 ml. Cool both solutions, then add the molybdate solution, slowly with stirring, to the sulfuric acid. When this solution has cooled dilute to exactly 1000 ml. with water.

Stannous chloride solution. Dissolve 25 gm. of $SnCl_2 \cdot 2H_2O$ in 100 ml. of concentrated hydrochloric acid, warming if necessary to dissolve. Dilute to 1000 ml. and filter if cloudy. Store in a container with a dropping device at the bottom. If a 10-mm. layer of white mineral oil is floated over the surface, the solution may be used for about 6 months.

Standard phosphate solution. Weigh 0.2195 gm. of recrystallized potassium dihydrogen phosphate into a 1000-ml. volumetric flask and dilute to volume with water. This is the base stock solution and contains 50 p.p.m. of phosphorus. Dilute 50 ml. of this solution to 500 ml. to obtain the standard solution actually used in the determination. This solution contains 5 p.p.m. of phosphorus.

Para-nitro phenol indicator solution. Dissolve 0.5 gm. of solid para-nitro phenol in 100 ml. of water.

Phosphorus-free hydrogen peroxide. Preparation from 30 per cent hydrogen peroxide is given under "Discussion of the Method."

Determination

Weigh in quadruplicate a 1 gm. sample of 35-mesh soil into a 500-ml. Erlenmeyer flask graduated at 200 ml. Each set of two is a duplicate determination. Add 15 ml. distilled (phosphorus-free) hydrogen peroxide to each of two of the flasks. Then add water until the total volume in all is 30 ml. Shake the flask thoroughly so that the soil is well mixed and wetted by the solution. Place the flask on the steam bath and turn the steam on high so that it flows freely around the bottom of the flask. Shake the sample again at the end of 15 minutes. After an additional 15 minutes remove from the steam bath and add about 100 ml. of water, then add 20 ml. of 2.0 *N* sulfuric acid, and finally make up to 200 ml. with water. Stopper, place in a shaker, and shake for 1 hour. Filter carefully, being sure that no trace of sediment occurs in the filtrate. Pipette a suitable aliquot into a 250-ml. beaker and evaporate on the steam bath until only a residue of sulfuric acid remains.[3] Dilute to 50

[3] The size of the aliquot will depend on the quantity of phosphorus in solution. For accurate results, the volume of standard solution to make the reading should approach the volume of the unknown. In this work an aliquot was taken which would give a final reading between 30 and 65. For example, an aliquot of 10 ml. serves for all soils containing between 300 and 650 pounds of phosphorus per acre when 5 ml. of standard solution is used.

ml. with water, add one drop of para-nitro phenol indicator solution and 1+1 ammonium hydroxide until the solution turns a faint yellow. Add just enough 2.0 *N* sulfuric acid to make the solution colorless again. This technic adjusts the pH to about 3.

Add 2 ml. ammonium molybdate-sulfuric acid solution, shake, then add 3 drops of stannous chloride and shake again. This solution is poured into a 100-ml. Nessler tube. The standard is made up as follows: pipette 5 ml. of a 5 p.p.m. phosphorus solution into a 300-ml. Erlenmeyer flask, add ammonium hydroxide and sulfuric acid to give the same amount of ammonium sulfate per milliliter as in the unknown, and adjust to pH 3 as before.[4] Dilute the standard to 96 ml. Since the standard is twice the volume of the unknown, 4 ml. of molybdate-sulfuric acid reagent and 6 drops of stannous chloride are added to it. The final volume of the standard is 100 ml. Pour the developed standard into a 100-ml. graduated cylinder. Hold both the Nessler tube containing the unknown and a similar empty one vertically over a white paper background.[5] Pour the standard solution into the empty Nessler tube until the colors of the two solutions match. The reading is the number of milliliters of standard required to equal the depth of color of the unknown.

This method of developing and reading the phosphate solutions is patterned after the one suggested by Truog (15), and his precautions should be observed.

Calculations

The organic phosphorus of a soil is calculated by subtracting the phosphorus in the unoxidized extract (easily acid-soluble phosphorus) from the phosphorus in the oxidized extract (organic + easily acid-soluble phosphorus).

Find the weight of soil represented in the aliquot taken. For a 10-ml. aliquot this is 0.05 gm. If 5 ml. of a 5 p.p.m. phosphorus solution was taken as the standard, it contains 0.25 p.p.m. of phosphorus. If the final reading is 50, the amount of phosphorus in the soil is equal to

$$\frac{50 \text{ (reading)}}{0.05 \text{ (wt. of soil)}} \times \frac{0.25 \text{ (conc. of standard)}}{1} = 250 \text{ p.p.m. of dry soil}$$

All results have been reported on the basis of pounds of phosphorus per 2,000,000 pounds of soil. This is obtained by multiplying the number of parts per million by 2.

[4] The amount of ammonium sulfate in the standard affects its color intensity and must be taken into consideration. A new standard is not required for each different-sized aliquot, but the comparison of an unknown, which needed only 5 drops of 1 + 1 NH_4OH for neutralization with a standard to which 40 drops of NH_4OH has been added, will cause an error. Sufficiently accurate amounts of ammonium sulfate can be formed in the standard solution if the drops of NH_4OH necessary to neutralize the acid of the unknown are counted, then twice this number of drops is added to the standard. Drop the acid into the solution until the color disappears.

[5] Since it is the volume of standard and not its actual depth that is read, it is necessary to obtain two uniform Nessler tubes of the same inside diameter, i.e., with the graduation marks at the same height.

DISCUSSION OF THE METHOD

The development of the method fell naturally into three parts: (*a*) preparation of a phosphorus-free hydrogen peroxide; (*b*) obtaining maximum decomposition of the organic matter and release of all the organic phosphorus in a soluble form; (*c*) accurate measurement of the phosphorus in solution after extraction. These points will be discussed in detail and the reasons for the various steps in the final procedure will be given.

a. Commercial grades of hydrogen peroxide all contain appreciable amounts of phosphorus, and even with the highest grade a blank would be necessary with each set. Consequently the peroxide used in this work was distilled under reduced pressure. A 500-ml. Claisen flask was fitted with ground glass connections, a bubbling tube to prevent bumping was inserted into the flask, and a thermometer was placed in a glass tube, which was set in the other opening. The delivery tube from the flask led to an all-glass Liebig condenser, and the distillate was collected in a suction flask connected to the source of vacuum through a mercury manometer and a water trap (pl. 1). The distillation was carried out under reduced pressure at a temperature below 60°C.

TABLE 1

Effect of concentration of peroxide on organic phosphorus values

SOIL	ORGANIC PHOSPHORUS VALUE			
	5 ml.* H_2O_2	10 ml.* H_2O_2	15 ml.* H_2O_2	20 ml.* H_2O_2
	lb./A.	*lb./A.*	*lb./A.*	*lb./A.*
S6755	350	360	360	360
S6757	440	510	590	590

* This volume of distilled peroxide was diluted to 30 ml.

Most of the liquid came over below this temperature, but because of decomposition and of the fact that the residue was more concentrated than the distillate, the distilled peroxide was not so concentrated as the undistilled. If the peroxide was 30 per cent at the beginning of the distillation it was usually about 20 per cent at the end. A stream of cold water was kept circulating through the condenser, and the distillate was collected under cold water. About 250 ml. of hydrogen peroxide was distilled at a time. Since the water bath around the flask was heated slowly, the time of distillation was 4 to 5 hours. A little calcium hydroxide was added to the peroxide before each distillation to insure a phosphorus-free product. A similar setup has been described by Baumann (4).

b. With the use of a small sample of soil an excess of peroxide is easily obtained. Table 1 shows that 15 ml. of distilled peroxide is necessary for maximum organic phosphorus values on soils high in organic matter but that much smaller quantities are sufficient on light-colored soils. With peats and other organic soils it may be necessary to halve the size of the sample and increase the volume of peroxide to 25 ml. for complete oxidation.

At the beginning of this work the peroxide was added to the dry soil and the solution evaporated to dryness on the steam bath. Appreciable fixation of the liberated phosphorus occurs with this technic, however, and reliable results cannot be obtained. It was thought that less fixation might occur in a liquid medium, and this was found to be true. When the original volume in the flask is 30 ml., some liquid always remains at the end of the 30 minutes on the steam bath. If the sample is diluted immediately, the acid added, and the extraction begun, the amount of fixation is negligible on most soils. The following experiment demonstrates this point. Thirty milliliters of distilled water was added to duplicate 1-gm. samples of soil, both of which were placed on the steam bath. At the end of 15 minutes 1 ml. of 50 p.p.m. phosphorus solution was added to one sample, and both flasks were shaken and returned to the steam bath for 15 more minutes. The usual procedure (p. 31) was then followed. The quantity of phosphorus added was the equivalent of 100 pounds per acre and, since it was water soluble, simulated the conditions when a soil containing 100 pounds per acre of organic phosphorus was treated with peroxide. If no fixation occurred the treated sample would give a value of 100 pounds of phosphorus per acre higher than that of the untreated. The difference between the two values, subtracted from 100 gave the pounds of phosphorus fixed per acre. Such an experiment gave complete phosphorus recovery with a majority of the soils studied, i.e., no fixation occurred. The highest amount of fixation, 20 of the 100 pounds of phosphorus added, took place in soils low in organic matter and high in active iron and aluminum. In such cases a correction factor may be applied to the organic phosphorus value as determined, and a more accurate value calculated. This fixation study would not have to be run on every sample. Cecil clay loam, an orange-colored lateritic soil from South Carolina, extremely low in organic matter, fixed 20 pounds of phosphorus per acre. We may consider this amount to be the maximum quantity likely to be fixed by most soils found in the United States. A gray silt loam surface soil (S6763), containing only 1.72 per cent organic matter, gave no fixation. It is suggested that a few preliminary trials would suffice to determine whether the soils of any given area fix enough phosphorus to warrant correction of the organic phosphorus values.

When the study was begun 0.002 *N* sulfuric acid buffered at pH 3 was used as the extracting agent. It was soon discovered that some of the phosphorus liberated during the oxidation was being fixed in a form which was insoluble in this dilute acid. Odynsky (8) recommends the use of 2.0 *N* sulfuric acid, but since it was found that 0.2 *N* sulfuric acid gave the same organic phosphorus values and is more satisfactory, as much lower quantities of iron were dissolved, this concentration was finally adopted.

The period of extraction was also investigated. Table 2 gives illustrative values showing that maximum organic phosphorus values are obtained when the soils are shaken for 1 hour, and this time was therefore used.

c. The measurement of the phosphorus in the filtrate was complicated by

the presence of peroxide, which interferes with the development of the blue color. The peroxide cannot be destroyed by boiling the solution to dryness, as phosphoric acid is more volatile than sulfuric acid and some phosphorus is lost. Various chemical and catalytic methods of peroxide decomposition were tried but were found to interfere with some other part of the determination. The procedure finally adopted consists of evaporation of the filtrate on the steam bath to a sulfuric acid residue. This treatment effectively destroys the peroxide without volatilization of phosphorus. It is recommended that all the solutions be heated on the steam bath to offset any difference in shade of color which might develop if only the ones containing peroxide were heated.

The hydrogen-ion concentration is an important factor to be considered in the development of the blue color, as pointed out by Truog and Myer (16). The method given about for controlling the pH was found satisfactory and is not too complicated.

TABLE 2

Effect of time of shaking on organic phosphorus values

SOIL	ORGANIC PHOSPHORUS VALUE BY EXTRACTION FOR		
	30 min.	60 min.	120 min.
	lb./A.	*lb./A.*	*lb./A.*
S6755	345	360	360
S6757	440	590	530

DISCUSSION OF RESULTS

The soils used in the development of this method were taken for the most part from check plots of experiment fields in various parts of Illinois. They vary widely both from the standpoint of classification and from the point of view of stage of development; that is, some were prairie soils with little profile development, high in organic matter and organic phosphorus; others had well-developed profiles and contained much lower quantities of these constituents. Well-developed forest soils were also included in this work, and sample S6769 is a subsoil from the latter group. Cecil clay loam provided a still wider variation in soils than could be found within the confines of this state. The final procedure gave reproducible values which were considered reliable, and it is believed that the method, perhaps somewhat modified to meet particular local conditions, is capable of accurately measuring the organic phosphorus of a wide variety of soils.

Table 3 gives the organic phosphorus values of the soils so far studied. It is seen that the quantity of organic phosphorus increases as the amount of organic matter increases, but, as would be expected, the factor obtained by dividing the organic matter by the organic phosphorus is by no means constant. It varies from 109 to over 200.

The phosphorus in the oxidized extract (organic plus easily acid-soluble inorganic phosphorus) of prairie soils with little profile development equals or closely approaches the total phosphorus (first four soils and peat in table 3). As shown by the other samples of table 3, in the well-developed forest soils and prairie soils with well-developed profiles, the phosphorus in the oxidized extracts no longer approaches the total phosphorus. It seems probable that these latter soils contain a form of phosphorus which is so insoluble that treatment with dilute acid leaves it unaffected.

Although the few soils so far investigated offer many interesting possibilities, further work is necessary before any interpretation of agronomic significance can be undertaken. Further work is necessary also before any clearer idea

TABLE 3

Organic phosphorus content and related data for 10 soils

SOIL	TOTAL PHOSPHORUS	P IN SOIL EXTRACT		ORGANIC P	ORGANIC P AS PER CENT OF TOTAL P	ORGANIC MATTER IN SOIL	ORGANIC MATTER / ORGANIC P	*	P FIXED
		Oxidized with H_2O_2	Unoxidized						
	lb./A.	*lb./A.*	*lb./A.*	*lb./A.*	*per cent*	*per cent*	*ratio*	*lb./A.*	*lb./A.*
S6758	1,475	1,425	440	985	67	7.83	159	540	0
S6757	1,330	1,310	720	590	44	6.10	207	820	0
S6765	615	630	205	425	67	....	...	305	0
S6761	800	640	255	385	48	3.70	192	355	0
S6767	980	585	175	410	42	3.32	162	275	0
S6755	710	470	110	360	51	1.97	109	210	0
S6763	595	415	120	295	50	1.72	116	220	0
S6769	645	210	110	100	15	0.67	134	190	20
Cecil	.....	80	50	30	..	....	...	130	20
Peat	1,985	1,900	860	1,040	57	....	...	950	10

* Phosphorus in the extract of soil which had been treated previously with 100 pounds per acre of phosphorus as KH_2PO_4; not treated with H_2O_2.

of the structure of the organic phosphorus compounds in the soil can be obtained. The relationship of the organic and inorganic phosphorus of soils is another subject of interest. Do soils tend to accumulate organic phosphorus, or is the organic phosphorus a reservoir which is gradually becoming depleted under the influence of cultivation? The development of this method is but the first step in the solution of these larger problems.

SUMMARY

A method has been described for the determination of the organic phosphorus of soils. It is based on the liberation of phosphorus by decomposition of the organic matter with hydrogen peroxide and subsequent extraction with 0.2 *N* sulfuric acid. The procedure is comparatively simple and rapid.

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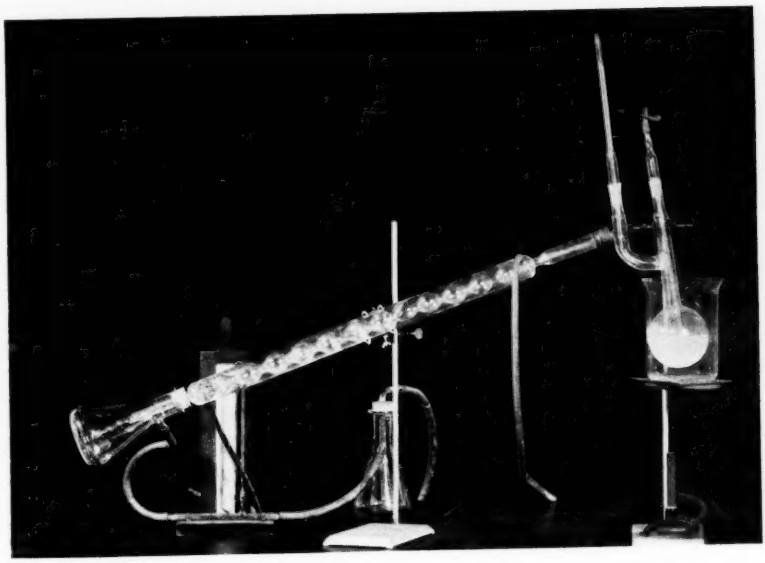
PLATE 1

PEROXIDE-DISTILLATION APPARATUS

Cooling bath for receiver not shown

PLATE 1



A SIMPLE METHOD OF ESTIMATING TOTAL SULFATES IN SOILS AND IRRIGATION WATER

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Salinity in soils consists mainly of the chlorides and sulfates of sodium. Estimation of chlorides presents no difficulty. The only method of sulfate estimation heretofore considered reliable, however, has been the gravimetric method, which is time-consuming. It was felt, therefore, that a volumetric method for estimating sulfates would be helpful in the examination of saline soils.

EXPERIMENTAL

The starting point of this investigation was the reaction $BaCO_3 + Na_2SO_4 \rightarrow BaSO_4 + Na_2CO_3$. It is obvious that if this reaction could be brought to completion sodium sulfate could be estimated by titrating as sodium carbonate. Preliminary experiments, however, showed that the forward reaction stops at a certain concentration of Na_2CO_3, because the solubility of $BaCO_3$ decreases with increasing concentration of Na_2CO_3 and becomes almost nil at the limiting value. It is necessary, therefore, to neutralize the Na_2CO_3 as it is produced. The success of the method, depends on the choice of a suitable indicator that will show a color change in the presence of Na_2CO_3 and be unaffected by the slight solubility of $BaCO_3$. Such an indicator is thymolphthalein, which gives a blue color in the presence of Na_2CO_3. In the presence of this indicator, therefore, when a solution of an alkali sulfate is shaken with $BaCO_3$ blue color is produced, which is discharged on the addition of acid until the whole of the alkali sulfate is converted into $BaSO_4$. As calcium salts would interfere in these titrations, any that are present must be removed by the addition of ammonium carbonate. Magnesium salts do not interfere.

DETAILED DESCRIPTION OF THE METHOD

Add solid ammonium carbonate to a portion of the solution and warm to 60°C. If no precipitate is formed, calcium salts are absent and the procedure then is as follows:

In a conical flask 50 to 100 cc. of the solution under test is boiled with 1 or 2 gm. of $BaCO_3$. A drop or two of a 0.5 per cent solution of thymolphthalein in alcohol is added. A blue color is developed at once if sulfates are present in the original solution. Then 0.1 *N* HCl is added in small quantities until

the blue color is discharged. The flask is again boiled until blue color develops, and more acid is added. The titration is thus continued until the solution remains colorless when boiled. As thymolphthalein color is likely to fade on standing, it is necessary to add one or two drops of the indicator now and again during the course of the titration, especially toward the end point. When the titration is complete the whole of the carbonate has been converted into bicarbonate, but the acid used cannot be taken as equivalent to half the alkali carbonate present, because overstepping is inevitable in a boiling solution because of the escape of carbon dioxide. The solution, therefore, is filtered and washed twice on the filter paper with hot water. The filtrate is then titrated with the same acid, methyl orange being used as indicator. The total acid used in the first and second titrations is equivalent to the alkali sulfate present in the volume of the solution under test.

If calcium salts are present, as indicated by the appearance of a white precipitate on the addition of ammonium carbonate followed by warming, add solid ammonium carbonate to an aliquot of the solution, warm to 60°C., and filter. The precipitate is washed twice with warm water, and the filtrate is evaporated to half the volume, all the free ammonia thus being driven out. Then 20 to 30 cc. of 0.1 *N* NaOH is added, and the boiling is continued for about half an hour. Thymolphthalein indicator is added, and the excess of sodium hydroxide is titrated against standard hydrochloric acid. The decrease in the concentration of the sodium hydroxide added is equivalent to the calcium salts present in the solution. The following reactions take place:

$$CaR_2 + (NH_4)_2CO_3 \rightarrow CaCO_3 + 2NH_4R$$

$$NH_4R + NaOH \rightarrow NH_4OH + NaR$$

After the preliminary separation of calcium salts, the sulfates in the filtrate are estimated by the method previously described. If one is not interested in the estimation of calcium salts the NaOH added need not be standard; 2 or 3 cc. of approximately *N* NaOH solution may be added, and the solution, after being boiled, can be made neutral to thymolphthalein by the addition of acid. As no extra labor is involved in working with standard solutions, however, it is generally worth while to gather this additional information.

The method described here has been found very useful for estimating sulfates in saline-alkali soils. Obtaining a clear filtrate from such soils is generally difficult. Filtration through porous candles under pressure is the most efficient method, but it is extremely cumbersome. We have had very satisfactory results in extracting with normal ammonium carbonate. The soil is flocculated and can be filtered through ordinary filter paper. Another advantage of ammonium carbonate solution lies in the fact that exchangeable sodium, which is a normal feature of such soils, is determined in the same filtrate.[1]

[1] Puri, A. N. 1935 Estimation of replaceable Na and K, exchange capacity, and degree of alkalization in alkali soils by ammonium carbonate extraction. *Soil Sci.*, 40: 249–253.

Twenty grams of soil is shaken with 200 cc. *N* $(NH_4)_2CO_3$ for 2 hours and filtered. One hundred and fifty cubic centimeters of the filtrate is evaporated to dryness. The residue is taken up with hot water and is filtered. The filtrate is made up to 100 cc. Half of the filtrate is titrated for total alkalinity, methyl orange being used as indicator. This value is equivalent to exchangeable sodium and potassium in the soil. Hydrochloric acid equivalent to this alkalinity is added to another aliquot of the filtrate, which is then titrated for sulfates after the addition of $BaCO_3$ according to the method described here.

TABLE 1

Estimation of sulfates in sodium sulfate solutions

0.1 *N* SULFATE ACTUALLY PRESENT	0.1 *N* SULFATE DETERMINED
cc.	*cc.*
1.0	1.6
2.0	2.6
4.0	4.4
7.5	7.7
12.0	12.7
30.0	29.9
40.0	40.0
50.0	49.9
60.0	60.1
70.0	69.6
80.0	79.9
90.0	89.8
100.0	99.1

TABLE 2

Estimation of sulfates in the presence of magnesium sulfate and calcium sulfate

0.1 *N* SULFATE ACTUALLY PRESENT		0.1 *N* SULFATE DETERMINED	0.1 *N* SULFATE ACTUALLY PRESENT		0.1 *N* SULFATE DETERMINED
$MgSO_4$	Na_2SO_4		$CaSO_4$	Na_2SO_4	
cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
1.0	10.0	11.3	2.0	10.0	12.6
2.0	10.0	11.8	4.0	10.0	14.2
3.0	10.0	12.5	6.0	10.0	15.7
4.0	10.0	13.7	8.0	10.0	17.3
5.0	10.0	15.3	10.0	10.0	18.8

If the soil under examination contains large amounts of calcium salts, they are eliminated in the ammonium carbonate extraction method; but such soils are in a flocculated state, and a clear solution can be obtained without the use of ammonium carbonate. A large excess of calcium salts also precludes the possibility of the soil's having much exchangeable sodium. A water extract of such soils therefore could be treated with ammonium carbonate if the estimation of calcium salts is of interest, and if not, the soil could be extracted

with ammonium carbonate, and the sulfates and chlorides estimated in the extract.

TABLE 3

Estimation of sulfates in the presence of calcium chloride

(10 cc. 0.1 *N* $CaCl_2$ in each 150 cc. of Na_2SO_4 solution)

0.1 *N* SULFATE, VOLUMETRIC METHOD	0.1 *N* SULFATE, GRAVIMETRIC METHOD
cc.	*cc.*
1.20	1.50
2.30	2.42
4.30	4.37
6.20	6.66
8.50	8.50
10.60	10.20
21.00	20.28
29.80	31.40
39.60	40.24
49.60	50.28

TABLE 4

Estimation of sulfates in soils

SOIL NUMBER	SULFATE	
	Volumetric method	Gravimetric method
	per cent	*per cent*
1	0.85	0.85
2	0.56	0.51
3	1.82	1.45
4	4.29	4.08
5	0.49	0.43
6	0.93	0.81
7	1.99	1.61
8	2.13	1.90
9	1.16	1.41
10	1.26	1.19
11	0.40	0.41
12	1.41	1.36
13	0.69	0.78
14	0.95	1.00
15	0.19	0.20
16	0.11	0.08
17	0.06	0.06
18	0.10	0.07
19	0.53	0.56
20	1.28	1.28
21	16.38	16.04

The limits of accuracy of the titration method can be judged from the results given in tables 1 to 4. In table 1 the results of sulfate estimation in sodium

sulfate solutions of known strength are recorded. In table 3 the effect of $CaCl_2$ on the sulfate estimate is shown. Each 150 cc. of the Na_2SO_4 solution of increasing strengths contained 10 cc. of 0.1 *N* $CaCl_2$. The calcium was precipitated by the addition of ammonium carbonate followed by filtration, and the sulfate was estimated in the filtrate after boiling. Table 4 gives the results of sulfate estimation in a number of soils. The estimations were made in the water extract, and calcium was precipitated where it was indicated.

The data in tables 1–4 show that the proposed method is extremely promising and should prove very useful for the examination of saline soils or salt efflorescence.

Results in table 2 show that magnesium or calcium sulfates do not interfere in the estimation. The calcium and magnesium sulfates are converted to carbonates by the addition of ammonium carbonate, and the liberated sulfate ions are ultimately converted to sodium sulfate and are estimated in the usual manner. The reactions may be illustrated as follows:

$$CaSO_4 + (NH_4)_2CO_3 \rightarrow CaCO_3 + (NH_4)_2SO_4$$
$$(NH_4)_2SO_4 + 2NaOH \rightarrow Na_2SO_4 + 2NH_4OH$$

SUMMARY

A rapid titration method for the estimation of total sulfates in soils and irrigation waters has been outlined.

The use of ammonium carbonate solution in extracting saline soils for the estimation of total soluble salts and sulfates is indicated.

A RAPID METHOD FOR DETERMINING THE PERMANENT WILTING POINT AND FOR INDICATING UNDER FIELD CONDITIONS THE RELATION OF SOIL MOISTURE THERETO[1]

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A practical and reliable method that would indicate quickly under field conditions how much available moisture is in the soil would be of great value, especially since from the standpoint of plant culture such information is more important than a knowledge of the total soil moisture content.

Although the method presented here does not indicate directly how much available water is in the soil, it does determine the permanent wilting point of the soil and indicates directly under field conditions whether the moisture is at, above, or below this point.

PRINCIPLE OF THE METHOD

The principle of the method is that at or above the wilting point the soil moisture film is sufficiently thick to cause the soil particles and granules to cohere when lightly pressed together, but below the wilting point the moisture film becomes too thin and discontinuous and is held with such great attractive forces that no such cohesion occurs. The change in the condition of the moisture film takes place within very narrow limits in the entire moisture range and is so pronounced that the determination of the wilting point by this method is reasonably accurate.

The principle of the method is well supported by established facts. The data in figures 1 and 2, as reported by Parker (3) and by Veihmeyer and Edlefsen (2) respectively for different soils, show that in the narrow range around the wilting point a pronounced change occurs in the relationship between the soils and their moisture contents, regardless of whether this change is measured by vapor pressure, freezing point depression, rate of evaporation, surface forces, or energy changes. The change in the curves must simply mean that the free water has ceased to operate and that the thin, discontinuous moisture films have come into play.

PROCEDURE

The following pieces of apparatus are required for the operation of the method:

A small 3-inch-blade spatula.

A base, painted with enamel paint, waxed or oiled, and polished so the soil will not stick on it.

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[1] Authorized by the director for publication as Journal Article 310 n.s. of the Michigan Agricultural Experiment Station.

A medium-sized 2-mm.-mesh sieve.
A small bottle of methyl alcohol. The neck of the bottle should be wide enough to permit the spatula to be dipped into the alcohol.
A half-pint Mason jar with cover.
An oilcloth about 12 inches square.
A medium-sized soil pan.
A 10-cc. pipette.

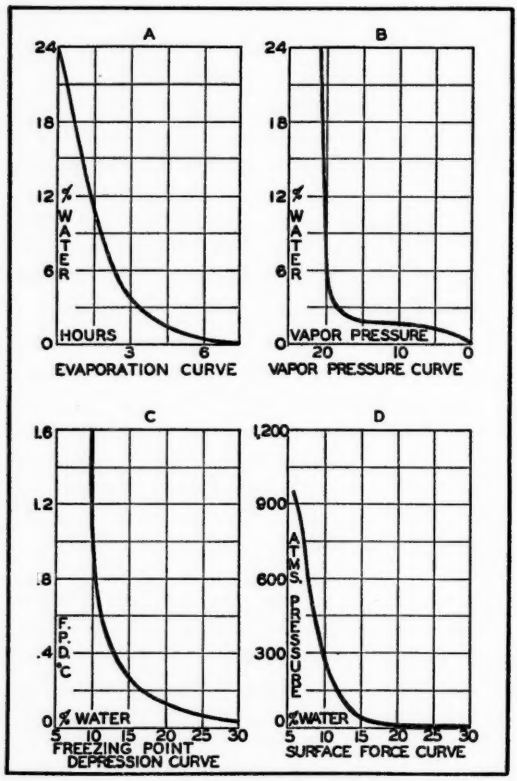

FIG. 1. CURVES SHOWING THE STRIKING CHANGE THAT TAKES PLACE IN THE RELATIONSHIP BETWEEN SOIL AND ITS MOISTURE WHEN THE WILTING POINT RANGE IS REACHED
Each curve represents a different soil [Parker (3)]

Two procedures in the method can be followed, depending on whether it is desired to ascertain, first, whether the moisture under field conditions is at, above, or below the wilting point, or, second, the wilting point of the soil.

For the first determination, the requisite field equipment comprises the base, spatula, screen, oilcloth, Mason jar, and bottle of alcohol. The procedure for this determination consists of taking a sample of the soil to be examined,

screening it rapidly on the oilcloth, mixing it, and putting it in the Mason jar. By means of the small spatula, about 1½ to 2 gm. of the soil is taken and placed on the base as a long mount or elongated pile and gently pushed on each side but not on the top so that the pile will be about as wide as the blade of the spatula and about ¼ inch high (pl. 1). Then, unless the soil is very wet, the spatula is dipped into the alcohol, shaken vigorously twice to throw off any excess alcohol, lightly pressed against the soil mass, and quickly lifted. If the soil moisture is at or above the wilting point the soil particles will stick to one another and to the spatula and will be lifted as a pressed soil mass or bar (pl. 1), but if the moisture is below the wilting point the soil particles will not cohere

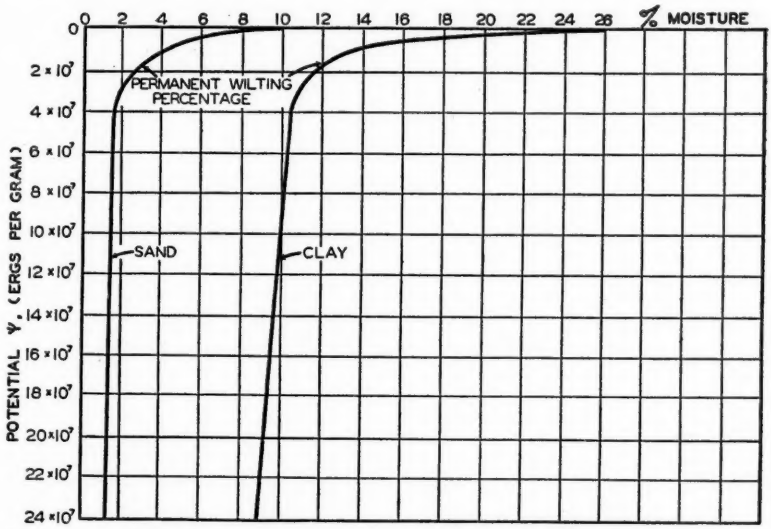

FIG. 2. CURVES SHOWING THE STRIKING CHANGE THAT TAKES PLACE IN THE RELATIONSHIP BETWEEN SOIL AND ITS MOISTURE WHEN THE WILTING POINT RANGE IS REACHED AND WHEN THE MOISTURE IS CONSIDERED FROM THE ENERGY RELATIONS STANDPOINT [N. E. EDLEFSEN (4)]

nor will they adhere to the spatula. When the soil is very wet it will stick to the spatula better without the alcohol than with it.

For determining the wilting point of soils in the laboratory by this cohesion method, the procedure is as follows: Approximately 50 gm. of an air-dry soil is weighed out and placed in a medium-sized soil pan. A definite amount of water is added to the soil, which is then mixed by hand, screened, mixed again, put into the Mason jar, and covered so it will not lose any moisture. The soil is then subjected to the sticking test already described. If the test is negative, i.e., if the soil particles do not stick to one another and to the spatula, more water is added and mixed with the soil. The procedure of

TABLE 1

The wilting point of soils as determined by the cohesion, the direct, and the dilatometer methods

SOIL DESIGNATION	NAME OF SOIL	PERMANENT WILTING POINT		
		Cohesion method	Direct* method	Dilatometer method
		per cent	*per cent*	*per cent*
CE	Columbia silt loam	9.03	8.61	8.62
OS	Delano silt loam	4.35	4.17	5.10
FAL	Farwell silt loam	14.2	14.14	14.39
FS	Fresno sandy loam	3.4	3.05	3.27
G	Columbia silt loam	7.1	6.69	6.97
H	Stockton clay adobe	8.9	9.28	9.31
HS	Harford fine sandy loam	4.2	3.99	5.27
I	Columbia sand	5.6	5.53	7.78
J	San Joaquin loam	6.7	6.34	5.67
K	Sierra sandy loam	6.1	5.09	5.65
L	Yolo fine sandy loam	8.9	9.11	9.56
M	Placentia loam	4.0	3.70	5.01
MG	Madera and Gridley loam	11.3	10.25	11.61
N	Okley fine sand	1.60	1.33	1.48
OC	Brockton clay	12.1	11.55	11.35
OL	Wooster silt loam	6.6	6.12	8.24
OS	Plainfield fine sand	1.9	1.36	1.79
S	Yolo silt loam	10.21	10.13	10.43
TL	Tehama loam	5.1	4.51	6.25
V	Catherine loam	20.60	19.03	19.75
Y	Yolo fine sandy loam	9.3	8.93	9.11
YC	Yolo clay	12.95	13.98	15.85
AK_2	Aiken clay loam	20.4	16.40	20.75
YS	Yuma sand	3.9	3.17	3.73
Z	Brazito fine sand	1.96	1.58	1.65
24	San Joaquin sandy loam	4.3	3.93	5.20
25	Madera sandy loam	4.92	3.62	5.19
	Davidson clay loam B	16.1		15.70
	McKenzie clay A	20.8		23.61
	Houston Clay A	20.3		20.91
	Catalpa clay B	14.8		16.75
	Ontonogon clay C	13.5		15.4
	Hagerstown silty clay loam	22.5		23.4
	Buchner silt loam, surface	14.4		14.4
	Clyde clay loam	25.6		27.4
	Nacogdoches fine sandy loam B	12.2		13.1
	Davidson clay loam B	16.1		15.7
	Muck	82.1		74.8
	Muck	83.5		77.2

* The permanent wilting point determinations by the direct method were made by F. J. Veihmeyer.

adding more water and mixing the soil thoroughly is continued until a positive test is obtained. At this point the total moisture is determined in the usual manner, and this moisture content represents the wilting point of the soil. The number of additions of water to the soil and the quantity of water to be added at the outset depend on the type of soil. If the soil, for instance, is sand, the wilting point of which may be about 2 per cent, only about 0.5 cc. of water is added at first to 50 gm. of soil. If the soil is loam, which has a wilting point of about 20 per cent, 7 to 8 cc. of water is added at first and 0.5 to 1 cc. after each test. In this manner the procedure can be carried out fairly rapidly.

This method has been tested on a large number of soils, and it has been found to be entirely satisfactory on all soils tested except heavy sticky clays, especially those from the lower horizons. It is difficult to distribute added water evenly on all the particles of such clays by screening; but if care is exercised the method will work satisfactorily even with the clays.

EXPERIMENTAL RESULTS

In table 1 are presented comparative results on the wilting point of a considerable number of soils as determined by the direct method, by the dilatometer method, and by the cohesion method. The comparison shows a close agreement among the results obtained by the three methods and indicates that the cohesion phenomenon is exceedingly sensitive to small changes in the moisture content when the wilting point range is reached. With the exception of the sticky clays and mucks, the method is sensitive to a few tenths of 1 per cent of moisture for most soils.

GENERAL DISCUSSION

From all the studies thus far conducted it appears that the cohesion method can determine the wilting point of soils accurately and can be used as a field method to tell quickly and simply whether the soil moisture is at, above, or below the wilting point. This method can be supplemented in the field by the burning alcohol method (2), which determines the total moisture content of soils accurately and rapidly.

The relationship of soils to water over the entire moisture range, as revealed by the curves on vapor pressure, freezing point depression, surface forces, and energy relations, supports the principle upon which the cohesion method is based. All these curves show that a rapid change occurs in the relationship of soils to water when a certain range of water is reached; and it happens that in this range the wilting point falls. It remains, therefore, only to measure this range or point by some practical method, and the cohesion method seems to be suitable for the purpose.

For the greatest success in applying the method, the following simple precautions and observations should be carefully noted:

The surface of the base, which may be of any convenient material, should be

made repellent to moisture. For this reason the surface should be painted with enamel paint, waxed or oiled, and polished.[2]

In pressing the soil the object is to bring the soil particles or granules into intimate contact with one another so that the moisture film will exert its cohesive force. It must be remembered that the cohesion that takes place in this test is due to the water film and not to the natural stickiness of the soil itself. It is not necessary, therefore, to press the soil hard. As a matter of fact, pressing too hard not only is fundamentally wrong, but also brings about undesirable results; when pressed too hard, silts, for instance, will not lift, and clays and other soils may lift prematurely or may stick to the base. The maximum pressure applied to light-textured soils is about 75–100 gm. as measured by the postal scale, and that applied to heavy sticky clays is about 100–200 gm. Because of their granular condition after being sieved, the clays must be pressed somewhat harder than the light soils. The technique of the pressing procedure is to hold the spatula with the index finger over the blade, to press lightly and quickly, and to lift quickly. When the right moisture content is reached the soil will press and lift easily and decisively, leaving the base directly under the spatula clean. Water in very small quantities should be added continually until this decisive result is obtained. Toward the last addition of water the soil should be left in the jar for a few minutes to allow the water to wet and to penetrate the soil particles thoroughly. When the wilting point is being approached, the soil begins to stick and to lift partially and irregularly. This is not the point at which to stop, but additional amounts of water should be added until the soil sticks and lifts in a clear-cut manner and the same result can be obtained repeatedly. When the soil reaches the true wilting point it ceases to be in a powder form, and its original color changes because of the thicker moisture film that is formed around the soil particles. After the soil reaches the wilting point, additional water does not increase perceptibly its sticking and lifting phenomena as measured by this method. Very little water need be added, therefore, after the soil shows signs of sticking and lifting. On the other hand, after the moisture has increased beyond the wilting point, the soil sticks to the spatula without the aid of the alcohol film. This simple test can be used as a check to ascertain whether the soil is too wet. In this particular check test, however, the soil must be sufficiently pressed. Many soils, as soon as they pass beyond the wilting point, fail to stick to the spatula if it has alcohol on it.

The object of dipping the spatula in alcohol is to form a thin film on the spatula so as to facilitate the adhesion and lifting of the soil mass. The spatula, therefore, should be shaken vigorously once or twice when taken out of the alcohol in order to throw off any excess alcohol. Around the wilting point range the aid of the alcohol film on the spatula is essential to the success of the test.

[2] A special base can be obtained from the Wood and Metal Work Co., Box 234, Bloomfield Hills, Michigan.

After the 50 gm. of soil is mixed with a definite amount of water and put into the Mason jar, any portion of the soil that is taken out for testing is returned to the jar when the test is completed. In this way one knows how much water to add for a definite increase in water content, be it 0.5 or 1 per cent.

The soil should always be passed through a 2-mm.-mesh sieve and thoroughly mixed so that the moisture will be well distributed throughout the mass. In addition, sieving is necessary because the soil mass must be in a loose and granular condition. When the method is used in the field, the sieving and mixing procedure should be done as rapidly as possible in order to avoid evaporation.

With the exception of very sticky clays, the method seems to work almost perfectly. It is sensitive and accurate with light-textured soils and is simple and rapid. With very sticky clays, especially those from the lower horizons, the method is not highly accurate. Because of the stickiness of these clays, it is extremely difficult to sieve them and thereby bring about a thorough and even distribution of the moisture on all their particles. As a result, a premature positive cohesive test may give somewhat lower wilting point readings. With care this method can be made to work satisfactorily even on sticky clays. For the stickiest clays, however, the dilatometer is the most accurate method for determining the wilting point (1).

In learning the technic of the method, it is best to start with sandy loams. The method works ideally on these soils, and from them one can determine readily the degree of pressure necessary and the sensitivity of the cohesion to small changes of moisture. Studies are in progress to devise an automatic means of pressing the soils and thus eliminate the personal element in the technic, but until this is developed the proper pressure of the spatula against the soil can be determined by practice on a postal scale.

When the soil, especially clay, is being wetted it indicates a slightly lower wilting point than it does when it is being dried by evaporation.

SUMMARY

A simple practical method is presented for determining the permanent wilting point of soils and for indicating under field conditions whether the moisture is at, above, or below this point.

The method is called the "cohesion method." Its principle is based upon the fact that when the moisture content of the soil is at or above the wilting point the moisture film around the soil particles is sufficiently thick to cause the soil particles or granules to stick to one another and to the spatula when the spatula is lightly pressed against the soil mass, and to lift on the spatula as a pressed soil bar. When the soil moisture, however, is below the wilting point the moisture film is too thin and discontinuous and is held by the soil with too great attraction to bring about these results. The cohesion that takes place in this test is due to the water films and not to the natural stickiness of the soil.

The principle of the method is well supported by the phenomena of vapor pressure, freezing point depression, rate of evaporation, surface force, and energy changes, wherein it is shown that the curves of those phenomena undergo a pronounced change in the region of the wilting point.

The cohesion method for determining the wilting point of soils has been compared with the direct method and with the dilatometer method, and it has been found to be exceedingly sensitive and accurate on practically all soils investigated except very sticky clays, the sieving of which and the resultant distribution of the moisture on all the soil particles of which are very difficult.

This simple cohesion method can also be used under field conditions to ascertain whether the field moisture is at, above, or below the wilting point.

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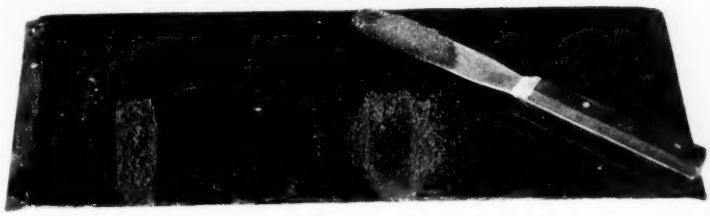
PLATE 1

Base, Spatula, and Pile of Soil Before Being Pressed and After Being Pressed, and Bar of Soil Lifted by Spatula



THE MEASUREMENT OF SURFACE AREAS OF SOILS AND SOIL COLLOIDS BY THE USE OF LOW TEMPERATURE VAN DER WAALS ADSORPTION ISOTHERMS

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In several recent publications (1, 3, 4, 5) we have described a method for measuring the surface of iron synthetic ammonia catalysts that involves the use of low temperature adsorption isotherms and have pointed out that apparently the method can be successfully used not only for metallic catalysts but also for other catalysts and catalyst-supporting materials such as chromium oxide gel, glaucosil, pumice, and pumice coated with nickel or nickel oxide. The success in these various cases suggested that the method might be useful for measuring the *relative* and perhaps the *absolute* surface areas of soils and soil colloids. To ascertain whether or not this is true, we have determined the adsorption isotherms that are reported in the present paper for two soil samples (Barnes soil and Cecil soil) and for the colloids of these two soils.[2]

It will perhaps be somewhat easier to understand the results obtained with the soils and soil colloids if the principle of the method is first illustrated by referring to results obtained with iron catalysts. In figure 1 are typical adsorption isotherms for nitrogen at $-195.8°$ and at $-183°$C., obtained on a singly promoted iron synthetic ammonia catalyst. It is evident that if one could select the point on such isotherms corresponding to the completion of a monomolecular layer of adsorbed molecules, a simple multiplication of the number of molecules in such a layer by the average cross-sectional area of each molecule would give the absolute area of the catalyst, subject only to the uncertainties in values for molecular diameters and in the closeness of packing of the adsorbed molecules. For iron catalysts the combined evidence from both physical and chemical adsorption data indicates that point B (figure 1), the beginning of the linear portion of the isotherms, marks the completion of a monomolecular layer. The evidence is threefold and can be briefly summarized as follows: 1. The surface areas of a particular iron catalyst calculated from the isotherms of several different gases close to their boiling points are more nearly constant if point B on the isotherms is considered to mark the completion of the monomolecular layer than if point A (figure 1) or one of several other possible points that need not be discussed here is chosen.

[1] Fertilizer Research Division, Bureau of Chemistry and Soils.

[2] Furnished by H. G. Byers and his associates in this bureau.

2. On pure iron catalysts one of the gases, carbon monoxide, forms instantaneously at −183° what appears to be a complete layer of chemisorption over the entire catalyst surface. The volume of this chemisorption agrees approximately with the volume of van der Waals adsorption at point B. 3. The heat of adsorption of nitrogen, as calculated from the −183° and −195.8° adsorption isotherms by means of the Clapeyron equation, undergoes a very rapid decrease with increasing volume of adsorption at about point B, being considerably greater (70 per cent or so) than the heat of liquefaction at a volume of adsorption slightly less than that at point B and only a little greater than the heat of liquefaction (15 per cent or so) at a volume perhaps 25 per cent greater than point B. [For a curve illustrating this, see (5, fig. 12).]

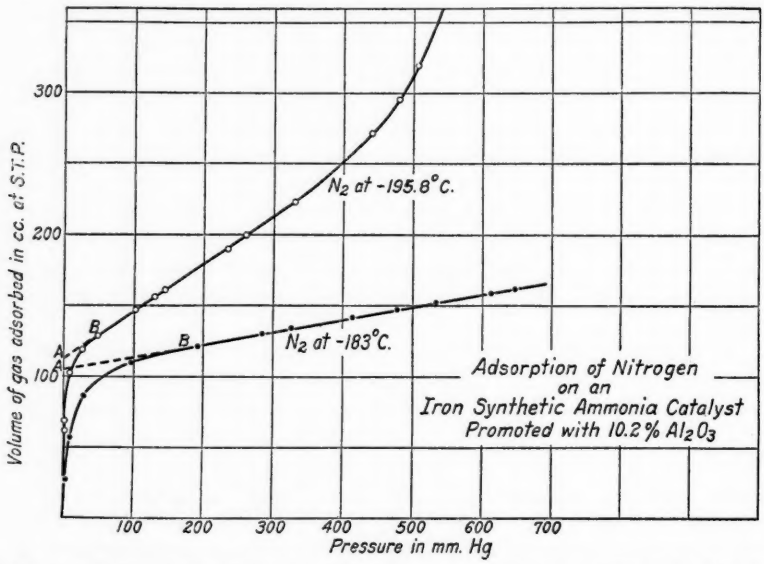

FIG. 1. ADSORPTION ISOTHERMS FOR NITROGEN AT −195.8° AND −183°C. ON AN IRON SYNTHETIC AMMONIA CATALYST PROMOTED WITH 10.2 PER CENT Al_2O_3 (CATALYST 954)

From these three types of evidence we have concluded that point B on the isotherms for iron catalysts represents, within an uncertainty of perhaps ±15 per cent, the volume of gas needed to form a monomolecular layer. The isotherms on chromium oxide, pumice, glaucosil, and similar non-ferrous materials are practically identical in shape with those obtained on iron. Since, as is well know, van der Waals adsorption depends but little on the chemical nature of the adsorbing substance, it has seemed permissible to assume that point B marks the completion of the monomolecular layer on the isotherms for these non-ferrous materials just as it apparently does for the iron catalysts. This same assumption will necessarily be involved in applying the method to

soils and soil colloids, the isotherms for which, as will be evident from figures 2, 3, 4, and 5, are very similar to those for iron.

The work on iron catalysts indicated that the volume of gas corresponding to point A, the intercept with the volume axis of the extrapolation of the linear part of the isotherm, was smaller than the volume at point B by a fairly uniform amount (about 15 per cent). It occasionally proves convenient to determine only three or four adsorption points on the linear part of the isotherm. In such instances point A is more readily determined than point B, and the surface area can then be determined fairly closely by assuming it to be 15 per cent larger than that calculated for point A.

EXPERIMENTAL

The soil samples consisted of Barnes soil and colloid No. 10308 and Cecil soil and colloid No. 9418. Their chemical and physical properties have recently been described by Byers, Alexander, and Holmes (2). As pointed out by these authors, the colloid samples consist of particles equal to or smaller than 0.3 μ. Air-dried samples were used so that the change in surface area resulting from the gradual removal of water by evaporation at 25° could be followed.

The gases used for the adsorption measurements were purified as previously described (3). Liquid oxygen was used for a low temperature bath (−183°). Adsorption measurements were made by standard technic, helium being used for dead space calibration. Isotherms of argon and nitrogen were made at −183° and of nitrogen and oxygen at 0°. A few runs were made with carbon dioxide at 0°.

RESULTS

The low temperature and 0° adsorption isotherms on the Barnes soil, Barnes colloid, Cecil soil, and Cecil colloid are shown in figures 2 to 5. Since the chief purpose of the present work was to ascertain whether the proposed method could be applied to soils, particular attention was directed toward determining the dependence of adsorption on mesh size, time of evacuation, and time allowed for equilibration. The results may be discussed conveniently under these several headings.

Effect of Mesh Size. In the adsorption experiments on colloids the portion not passing a 100-mesh screen was used, since it was found difficult to prevent the very finest colloid particles from being sucked throughout the apparatus during evacuation. A run was also made, however, on the portion of Cecil colloid sample that passed the 100-mesh screen in order to see whether the same adsorption would be obtained regardless of whether the colloid material was larger or smaller than 100 mesh. The sample that was larger than 100 mesh yielded an adsorption volume (point A) of 8.4 cc. per gram compared to a volume of 8.0 cc. per gram for the portion finer than 100 mesh. It therefore seems probable that the portion of the colloid caught on the 100-mesh screen

may safely be considered as representative of the colloid as a whole. For convenience, only that portion of the Barnes soil caught on a 14-mesh screen and that portion of the Cecil soil caught on a 40-mesh screen, were used. It cannot be said with certainty, therefore, whether the results for the Barnes and Cecil samples were characteristic of these respective whole soils, but it seems probable that such is the case since, we understand, the soil particles in heavy clay soils are likely to be rather uniform agglomerates of all the various clays, colloids, and larger mineral particles that are present.

Effect of prolonged evacuation of the sample. The influence of time of evacuation on the sorptive capacity of the samples is shown by the data included in the legend for each figure. Twenty hours' evacuation at 25° appeared sufficient to produce an approximately constant sorption capacity for the 25-cc. samples of Cecil soil and colloid, whereas about 90 hours' evacuation seemed necessary for the Barnes samples. Evacuation at 100° following that at 25° changed the sorptive capacities of the samples in a rather unexpected way. The sorptive capacities of the Barnes soil and colloid at −183° were lowered 25 and 11 per cent, respectively, by evacuation at 100 to 110° for 18 hours, whereas the sorptive capacity of the Barnes colloid for nitrogen at 0° actually increased 5 per cent. The Cecil colloid showed virtually no increased adsorption at −183° as a result of 17 hours' evacuation between 100 and 122° following the 25° evacuation, though the adsorption of nitrogen at 0° increased 25 per cent. The increase in sorptive capacity of the Cecil soil at −183° after 16 hours degassing at 100 to 111° does not exceed 12 per cent and may actually be somewhat smaller.

Rate of equilibration. Adsorption equilibrium of argon and nitrogen on the Barnes soil and colloid was rather slow, as much as 3 or 4 hours being required before the adsorption became essentially constant. The Cecil samples equilibrated much more rapidly, 15 minutes sufficing for the colloid.

DISCUSSION AND CONCLUSIONS

The absolute area that would be covered in close packing by the volume of nitrogen corresponding to point A on the isotherm for a gram of the Cecil soil is 22.8 sq. m., for the Cecil colloid, 41.3 sq. m., for the Barnes soil, 31.2 sq. m., and for the Barnes colloid 71.4 sq. m. These numerical values are obtained if one calculates the diameter of the nitrogen molecules from the density of solid nitrogen (5). Using molecular diameters calculated from the density of liquid nitrogen, one obtains absolute area values about 23 per cent larger than the above.[3]

The size of particles (considered as spheres) of density 2.8 required to yield the above surface areas is about 3.0×10^{-6} cm. for the Barnes colloid and

[3] By analogy with the results on iron, the volume of gas at point A is probably about 15 per cent smaller than that at B. Hence, the values given above for the areas corresponding to point A are probably about 15 per cent lower than the actual surface areas of the soils and colloids.

5.2×10^{-6} for the Cecil colloid. These values are in good agreement with the size of the crystallites of the clays in the soil colloids estimated from X-ray diffraction patterns to be between 10^{-5} and 10^{-6} cm. (7).

It should be made perfectly clear that the surface measured by the present method is not the combined surface area of the agglomerates of soil colloids but the sum of the surface area of each of the tiniest crystallites going to make up the agglomerates. Hence the particle diameters calculated above are likewise for the crystallites composing the agglomerates and not for the agglomerates themselves.

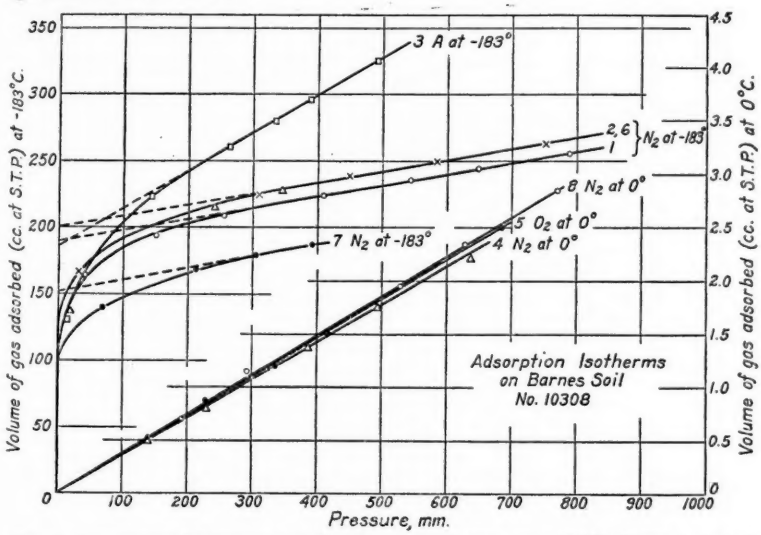

FIG. 2. ADSORPTION ISOTHERMS FOR NITROGEN AND ARGON AT −183°C. (RUNS 1, 2, 3, 6, AND 7) AND FOR NITROGEN AND OXYGEN AT 0° (RUNS 4, 5, AND 8) ON 24.2 GM. (25 CC.) OF BARNES SOIL NO. 10308 OF 8–14 MESH SIZE

Curves 1, 2, 3, 4, 5, and 6 were made after 18½, 31, 46, 57, and 103½ hours' evacuation at room temperature, respectively. Runs 7 and 8 followed about 19 hours' evacuation at 106–110°C. in addition to the preceding 103½ hours' evacuation at room temperature.

Thus far the discussion has been restricted entirely to the calculation of absolute surface areas. For reasons pointed out in connection with the work on iron, *relative* areas may be obtained from the low temperature adsorption isotherms with even greater accuracy than the *absolute* areas. It will be noted, furthermore, that the relative surface areas for two soils or soil colloids obtained by comparing point A on the respective −183° nitrogen isotherms are the same as those obtained when using the argon isotherms. This is evident from the isotherms in figures 2 to 5.

Gile and co-workers (6) in connection with their experiments on the adsorption of dyes, water vapor, and ammonia by soils and soil colloids have pointed

out that probably most of the adsorption capacity of a soil is centered in the colloidal fraction and that therefore the determination of the adsorption capacity of a soil and its colloid should enable one to calculate the percentage colloid in the original soil sample. It is interesting to note that the application of their method of calculation to the present adsorption results indicates that the Barnes soil contains about 43.7 per cent colloid. (The adsorption at point A being 8.34 cc. nitrogen per gram of soil and 19.1 cc.[4] per gram of colloid the percentage colloid is $\frac{100\ (8.34)}{19.1} = 43.7$.) This compares with 31.5 per

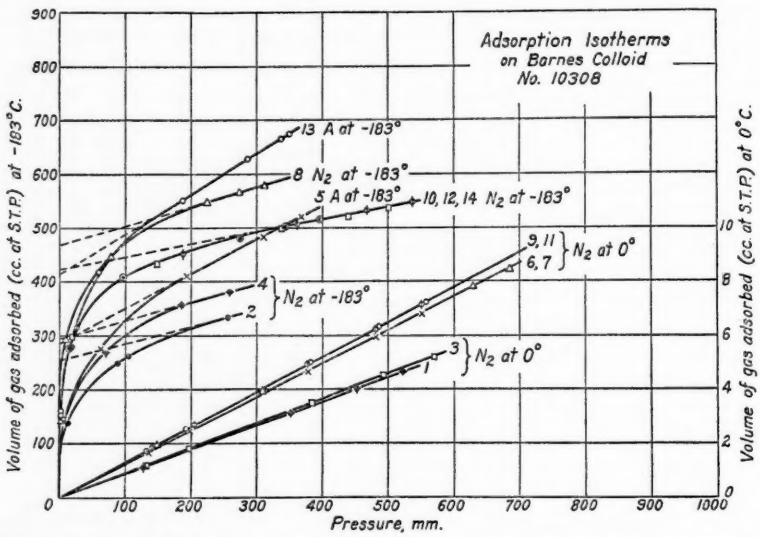

FIG. 3. ADSORPTION ISOTHERMS FOR NITROGEN AND ARGON AT −183°C. (RUNS 2, 4, 5, 8, 10, 12, 13, AND 14) AND FOR NITROGEN AT 0° (RUNS 3, 6, 7, 9, AND 11) ON 24.6 GM. (25 CC.) OF BARNES COLLOID NO. 10308 OF 10–100 MESH SIZE

Curves 1, 2, 3, 4, 5, 6, 7, and 8 were made after 7½, 19, 20, 21, 22, 85, 104, and 105 hours' evacuation at room temperature, respectively. Runs 9 to 14 inclusive were made following 17½ hours' evacuation at 100–110° in addition to the 105 hours' evacuation at room temperature.

cent colloid reported from mechanical analysis as being smaller than 0.002 mm. and 41.2 per cent smaller than 0.005 mm. A similar calculation for the Cecil soil indicates 55.2 per cent colloid on the basis of the surface measurements here reported, compared to 44.2 per cent colloid less than 0.002 mm.

[4] A 5-cc. (5.04-gm.) sample of Barnes colloid gave an adsorption at point A of 22.7 cc. per gram after 92 hours' evacuation and 23.3 cc. per gram after 180 hours. These results would indicate about 35 per cent colloid rather than the 43.7 per cent reported above. The cause of this discrepancy was not further investigated, though it seems probable that it may arise from the relatively greater efficiency of evacuation per unit pumping time on a 5-cc. sample than on a 25-cc. sample.

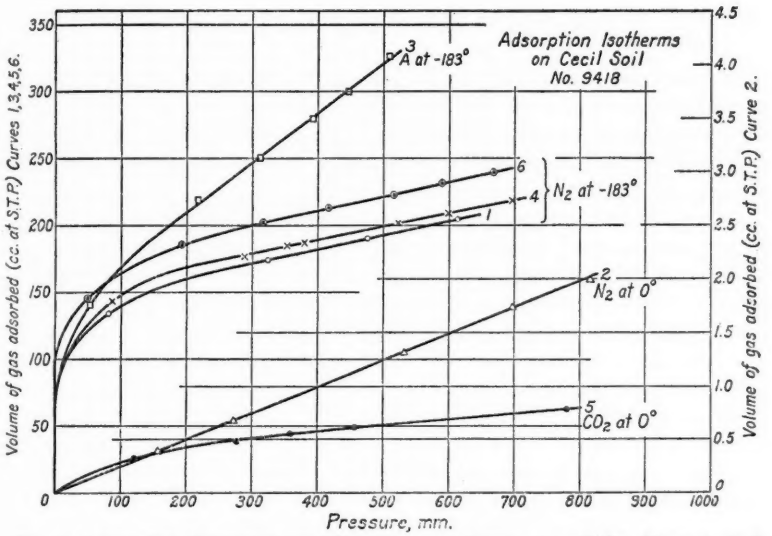

FIG. 4. ADSORPTION ISOTHERMS FOR NITROGEN AND ARGON AT −183°C. (RUNS 1, 3, 4, AND 6) AND FOR NITROGEN AND CARBON DIOXIDE AT 0° (RUNS 2 AND 5) ON 24.6 GM. (25 CC.) OF CECIL SOIL NO. 9418 OF 20–40 MESH SIZE

Curves 1, 2, 3, 4, and 5 were made after 18, 35, 53, 74, and 134 hours' evacuation at room temperature, respectively. Curve 6 followed 16 hours' evacuation at 111° in addition to the 134 hours' evacuation at room temperature.

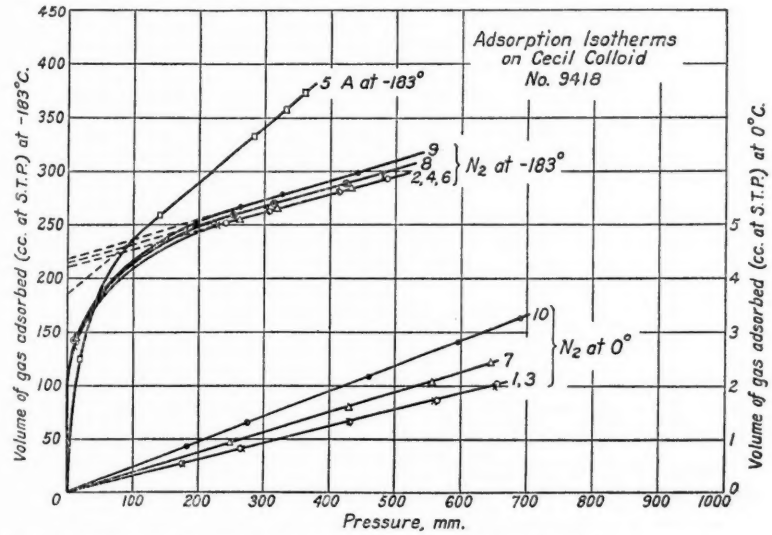

FIG. 5. ADSORPTION ISOTHERMS OF NITROGEN AND ARGON AT −183°C. (CURVES 2, 4, 5, 6, 8, AND 9) AND OF NITROGEN AT 0° (CURVES 1, 3, 7, AND 10) ON 19.9 GM. (25 CC.) OF CECIL COLLOID 9418 OF 20–100 MESH SIZE

Curves 1, 2, 3, 4, 5, 6, 7, and 8 followed 18½, 20, 21, 22, 23, 24, 81, and 82 hours' evacuation at room temperature; curves 9 and 10 followed 17 hours' evacuation at 100–122° in addition to the 82 hours' evacuation at room temperature.

in diameter and 51.8 per cent smaller than 0.005 mm. according to the results obtained from mechanical analysis. It seems therefore that the surface area measurements reported in the present paper are in full accord with the prediction of the aforementioned authors that most of the sorptive capacity of soils would be centered in the colloid fraction.

Because of the somewhat greater ease and rapidity of measuring adsorptions at 0° than at −183°, it was hoped that the volume of nitrogen found to be required to form a monomolecular layer on the soil would bear some constant ratio to the volume of nitrogen adsorbed at 0° at some pressure—say 760 mm. The results shown in figures 2 to 5 make it clear, however, that this ratio is not constant; it varies between 70 and 42 for the Barnes soil and colloid at various stages of degassing and between 75 and 61 for the Cecil soil and colloid.

The present data are insufficient to warrant attempting an extended correlation with the other known physical and chemical properties of these soil samples. Accordingly, an accurate assessing of the value of the proposed method of surface measurement as a tool for studying soils and soil colloids may appropriately be left to soil chemists and physicists who are familiar with this field of work and who may choose to extend the present work to include a much larger number of soils. Sufficient work has been done, however, to make it appear probable that no insurmountable difficulties are inherent in the application of the method to soils and soil colloids.

In conclusion, without entering into a detailed and critical discussion of all previous adsorption measurements on soils, we should like to point out that the method proposed in the present paper for measuring the absolute and relative areas of soils and soil colloids has two rather novel features. In the first place, the selection of the volume of adsorbed gas required for a monomolecular layer is much better substantiated in the recent work than in previous work because of the similarity between the isotherms for soils and those for iron catalysts. Secondly, the use of low temperature (−183°) isotherms of such gases as nitrogen and argon involves no specific chemical adsorption between the gas and the soil. This is certainly not true of adsorption measurements using water vapor, and may not be true of those using ammonia or carbon dioxide.

SUMMARY

Adsorption isotherms for nitrogen and argon at −183° and for N_2, O_2, and CO_2 at 0° are presented for samples of Barnes soil and colloid 10308 and of Cecil soil and colloid 9418 (2). By applying the same interpretation to these isotherms that was applied previously to similar isotherms for iron synthetic ammonia catalysts, values for the absolute as well as the relative surfaces of the soil and soil colloid samples were obtained.

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A RAPID METHOD FOR DETERMINING CARBON IN THE CARBOHYDRATE AND PROTEIN COMPOUNDS IN PLANT TISSUE[1]

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All standard methods for determining carbon are so long and complicated as to setup and manipulation that a large number of determinations for total carbon in plant tissues are out of the question. The only colorimetric methods found were one for carbon in steel by the use of nitric acid, which has not proved very satisfactory, and one which measures carbon dioxide from wet combustion.[2] The latter takes considerable time and necessitates the handling of gases. A great need exists for a direct rapid method for determining carbon in carbohydrate and protein compounds commonly found in plants.

When a carbohydrate or protein is heated with sulfuric acid, carbonization takes place, and the depth of color of the liquid is sensibly proportional to the amount of carbon present. Inasmuch as virtually all the carbon of plant tissue, especially vegetative tissue, is in the form of carbohydrate and proteins, and the proportion of carbon compounds that are not charred, such as oxalic acid or stable aromatic compounds, is very small, the color produced by charring with sulfuric acid should be a good index of the amount of carbon in the carbohydrates and proteins present. If it is known that nothing but carbohydrate or protein or substances which are completely charred are present it is an accurate method for determining total carbon.

After complete carbonization the solution is diluted with 50 per cent H_2SO_4 and compared to a standard in a colorimeter. The colloidal carbon solutions compare very well and give sharper end points than some colored true solutions.

CARBON IN SOLID SAMPLES

Put 50–100 mgm. of dry tissue or 250 to 500 mgm. of green tissue into a 200-cc. Erlenmeyer. Add 50 cc. (100 cc. for high C samples) of 50 per cent by volume H_2SO_4. Heat with a strong flame until all particles are carbonized thoroughly and the acid starts to condense on the sides (5–10 minutes). Do not heat past this stage, because after the acid carbonizes the sample it will

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

[2] Emmert, E. M. 1933 Rapid colorimetric determination of total carbon and nitrogen in the same sample. *Jour. Assoc. Off. Agr. Chem.* 16: 424–427.

start to oxidize the carbon at higher temperatures and cause a color change. Do not heat the sides of the flask above the liquid. Allow to cool. Make the volume to exactly 50 cc. with 50 per cent H_2SO_4 or to 100 cc. if C is high. Mix the solution thoroughly by shaking and pouring back and forth. If the color is still much darker than the standard, dilute an appropriate aliquot still further with 50 per cent H_2SO_4, taking care to mix well, allow to cool to about room temperature, and compare to a standard (0.25 to 0.125 mgm. C is best) in the colorimeter. Cheap technical H_2SO_4 may be used for diluting, but acid free of carbon must be used for the initial digestion.

TABLE 1

Carbon content of dry plant materials

SAMPLE	NUMBER	CARBON	
		Found	Theoretical
		per cent	*per cent*
Celophane	1	44.34	44.44
	2	44.36	
Whatman no. 40 filter paper	1	43.86	44.44
	2	43.80	
Rochelle salt	1	17.01	17.02
	2	17.00	
Potato starch baker, dried at 65°C	1	41.67	44.44
	2	41.67	
Gluten from wheat flour	1	54.80	55.00
	2	55.17	
Dried tobacco	1	31.25	
	2	30.30	
Dried green oat plants	1	33.10	
	2	33.10	

Preparation of Standard. Heat 5 cc. of 0.25 per cent glucose solution with 25 cc. of 50 per cent by volume H_2SO_4 until white fumes come off and acid begins to condense on the sides of the flask. Allow to cool to room temperature, make to exactly 20 cc. with 50 per cent H_2SO_4, and mix thoroughly. One cubic centimeter contains 0.25 mgm. of C. If 0.125 mgm. is desired, dilute to 40 cc. with 50 per cent H_2SO_4. The standard should be made up fairly close to the time the unknowns are tested.

Notes. Any tendency to off tints may be due to the standard's being considerably cooler than the unknown or to the standard's having stood longer. The unknown solutions should be made up fairly close to the time the standard

was made. Heat and time cause slightly different shades to be produced. The strength of the acid must be at least 50 per cent, or decided changes in tint result, and if the acid concentration is considerably weaker than 50 per cent, carbon may precipitate. Heating longer than to the point where white fumes come off freely and acid starts to condense may also change the color tints somewhat. For accurate work it is necessary to use precautions to keep color tints as near alike as possible.

If the C is high and the digestion volume of acid is small, carbon tends to precipitate. For this reason the digestion volumes should be increased on

TABLE 2

Carbon content of green tomato tissues

SAMPLE	NUMBER	C	TREATMENT OF THE PLANTS
		per cent	
Mature petioles near bottom of plants	1	3.20	Plenty of moisture
	2	3.29	
Tip petioles	1	3.63	In a moist soil (medium nitrogen)
	2	3.57	
Tip petioles	1	1.93	In a very moist soil (high nitrogen)
	2	2.27	

TABLE 3

Carbon content of pure glucose and sucrose

SUGAR ADDED	C ADDED	C FOUND
	mgm.	*mgm.*
0.5 cc. of 0.5 per cent sucrose	1.053	1.053
0.5 cc. of 0.25 per cent sucrose	0.527	0.527
0.5 cc. of 1 per cent sucrose	2.106	2.093
0.5 cc. of 0.1 per cent sucrose	0.211	0.210
0.5 cc. of 2 per cent sucrose	4.212	4.228
0.5 cc. of 0.5 per cent glucose	1.000	0.984
0.5 cc. of 0.5 per cent glucose	1.000	0.998
0.5 cc. of 0.25 per cent glucose	0.500	0.495

high C samples. It is well to keep the relation of digestion volume and carbon about the same as in the standard, or 5 cc. of digestion acid for each milligram of C. If nothing is known about the percentage of C in the unknown it may be necessary to do a little preliminary testing to determine the amount of 50 per cent acid to use for the digestion.

CARBON IN EXTRACTS OR SOLUTIONS

If the percentage of carbohydrates is high, a small aliquot can be treated as for carbon in solids, since water will boil off and the carbohydrate will be

carbonized. If the percentage of carbohydrate is low, a smaller amount of concentrated sulfuric acid should be used, and the dilution volume should be kept smaller in proportion to the carbon present. For very weak solutions a large aliquot should be used with 2 cc. of concentrated sulfuric acid, and the water should be boiled off and the heating continued until acid condenses. Dilute to an appropriate volume with 50 per cent H_2SO_4 and compare to a standard containing 0.25 mgm.; 0.125 mgm. or less may be used if the amount of carbon in the unknown solution is very small.

RESULTS WITH SOLIDS

Table 1 presents results from different types of dry plant materials.

The potato starch was a good grade of Baker starch, but the analysis was not given, and, undoubtedly, the sample contained some impurities. Absolutely pure starch is hard to obtain.

Citric acid and salicylic acids would not char enough to give a color. Strychnine sulfate charred to a great extent but would not give the theoretical percentage of carbon. The fact that tartaric acid charred readily shows that the method will determine the carbon in compounds of the carbohydrate type and where OH radicals are present. In the case of citric acid the one OH radical must be protected by the two other carboxyl groups which do not contain OH radicals. OH radicals in the benzene ring are unattacked by the acid.

Table 2 presents results with green tomato tissues and shows that the method detects differences in carbon content of tissues as a result of variations in soil moisture and nitrogen.

Table 3 presents results obtained with solutions of pure glucose and sucrose.

RESULTS WITH FATS

The method for total carbon was tried on fatty compounds to see what happens to the carbon they contain. Trials with palmitic acid would give no color, showing that straight chain compounds of the fatty acid series are not attacked by the procedure outlined here. Oleic acid gave some color but not nearly theoretical amounts. It is almost impossible to obtain oleic in an unoxidized condition because of its unsaturated property. Probably the acid attacked oxidized portions of the double bond linkage. Glycerol was readily and completely carbonized, as shown by the fact that the C found was 39.06 per cent and the theoretical is 39.10 per cent. This again shows that the action of the sulfuric acid is almost entirely confined to compounds in which hydroxyl groups are present.

BOOK REVIEWS

Solon Robinson—Pioneer and Agriculturist, vol. 2, 1846–1851. Edited by HERBERT ANTHONY KELLAR. Indiana Historical Bureau, Indianapolis, Ind., 1936. Pp. xvii + 556, ills. 13.

This is the second volume of a most interesting record relating to the agricultural history of the United States in the nineteenth century. The first volume covered the period 1825–1845: the present volume has to do with the period 1846–1851. The material presented here was compiled from articles by Robinson which appeared in a number of current publications, such as *The American Agriculturist* and *The Prairie Farmer*. The editor, however, has included in the material here presented writings that appeared in a considerable number of other publications, such as the *Daily Cincinnati Gazette, Western Ranger, Indiana State Sentinel, Farmer and Mechanic, National Intelligencer, American Farmer, De Bow's Review, Richmond Enquirer, Southern Cultivator, The Plow, Northern Almanac*, and the *Planters Pictorial Almanac*.

The reader cannot but be impressed with the great variety of topics dealt with by Robinson in his prolific writings. He visited many places, interested himself in nonagricultural as well as agricultural subjects, and acquired familiarity with the wide range of the contemporary agricultural enterprises and activities. To indicate the author's versatility, we may note that he treated of such subjects as land owned by Ewings in Lake and Porter Counties; a new arithmetical sum; Mark Cockrill's sheep farm; fences and other matters; practical facts about pork and bacon; a cheap farm house; warming houses with hot air and stoves; a lecture upon the early history of Lake County, Indiana; a rambling letter upon geology and other matters; western agriculture—corn cobs; formation of agricultural associations—importance of general diffusion of agricultural information; free homesteads; comment on route of railroad from Michigan City to Chicago; choice of trees and shrubs for cities and rural towns; cheese making; plantation tools; ventilation essential to health; and an agricultural tour south and west. (This pertains to an interesting journey in Indiana, Illinois, and Michigan. Another report has to do with a tour from St. Louis to Vicksburg, Miss.) In his other writings, he records the pumpkin dance and moonlight race; a visit to General Zachary Taylor at New Orleans; wintering in Louisiana and Mississippi; and reference to sugar cane. He wrote about the high water in the lower Mississippi, prospect of an overflow, etc.; frost and snow at New Orleans; recipes for ladies; Alabama wheat; facts in natural history; manufacturing in the South; and a long list of other topics. Altogether, this indefatigable traveler has recorded facts and observations which represent a substantial and extremely valuable contribution on American agriculture.

Moisture and Farming in South Africa. By W. R. THOMPSON, Central News Agency, Ltd., South Africa, 1936. Pp. 260, illus. 34. Price 21/–. London agents—Gordon & Gotch, Ltd.

It is widely recognized that water is the limiting factor in crop production even in regions where the rainfall ranges well up to 50 inches per annum. It is more of a limiting factor in the great wheat-growing areas, where the rainfall averages about 30 inches per annum. For this very reason, systems of crop and soil management have laid stress on moisture conservation and its efficient use in the production of economic plants.

In his foreword, Hubert D. Leppan, who is connected with the University of Pretoria, as is also the author, notes:

> From its inception, the Faculty of Agriculture, giving recognition to the truism that the more closely the farming of a country conforms with the dictates of the physical controls the more likely it is to succeed, has focussed much of its research on the climatic influences governing the rural development of the Union. It is perhaps not surprising, then, that Mr. Thompson, a former student of the Faculty of Agriculture, should devote himself to a study of the relationship between moisture supplies and South African farming. Certainly the correlation between the proper utilisation of available rainfall and success in the agricultural industry of theUnion is a close one.

The book contains 11 chapters, appendix tables, bibliography, and index. The chapters are designated, respectively: Introductory; The Alleged Drying up of South Africa and the Amelioration of the Drought Problem, with Special Reference to the Schwarz Kalahari Scheme; Historical Evidence in Connection with the Alleged Drying up of South Africa; A Study of South African Rainfall, Secular Variations and Agricultural Aspects; Rainfall Intensity, with Special Reference to Major Trends; Rainfall, Soil Erosion and Run-off in South Africa; The Rôle of Evaporation in the Dissipation of Moisture; Moisture Dissipation through Transpiration; The Rôle of Percolation in the Dissipation of Moisture; Veld Burning: Its History and Importance in South Africa; and General Discussions and Conclusions.

The reader of this book will find many parallels in the agricultural problems of South Africa to those in the United States, in Canada, South America, and Eastern Europe. Climatic changes, rainfall intensity and distribution, the relation of run-off to soil erosion and soil conservation, the burning of the vegetation of the plains, and similar topics are among the major considerations of world agriculture. The author has rendered a distinct service to the agronomist, the climatologist, and the agricultural economist in presenting data which should be useful in interpreting agricultural conditions and phenomena as they occur in many agricultural regions.

Methods of Statistical Analysis, ed. 2. By C. H. GOULDEN. Burgess Publishing Company, Minneapolis, Minn. Pp. iii + 209, tables 69. Price $3.00.

The author, who is the Senior Cereal Specialist of the Dominion (Canada) Rust Research Laboratory and Honorary Lecturer in Mathematics at the

University of Manitoba, has concerned himself in the second edition of his treatise with material which, in large measure, falls within the field of agricultural economics. In his preface to the second edition, the author says:

> Since the advent of R. A. Fisher's "Design of Experiments" and recent papers by F. Yates a good deal of attention is being given to the possibilities of improving experimental technique by means of the procedure known as confounding. Applied at first in factorial experiments it gave excellent results and recently F. Yates has made an extremely valuable application of the principle to tests involving a large number of varieties. These two aspects of confounding have been dealt with in this edition under the chapter on The Field Plot Test.

The treatise contains 15 chapters and an appendix. The chapters are designated, respectively: Introduction; Frequency Tables—Mean and Standard Deviation; The Normal Frequency Distribution; Tests of Significance with Small Samples; The Correlation Coefficient; Linear Regression; Partial and Multiple Correlation; The X^2 Test for Goodness of Fit and Independence or Association; Tests of Goodness of Fit and Independence with Small Samples; The Analysis of Variance; The Field Plot Test; The Analysis of Variance Applied to Linear Regression Formulae; Non-Linear Regression; The Analysis of Covariance; and Miscellaneous Applications.

The material here presented should be most helpful to the geneticist, the biochemist, the agronomist, and others who find themselves concerned with the statistical analysis of all sorts of experimental and survey data.

Organic Chemistry. By FRANK C. WHITMORE. D. Van Nostrand Company, Inc., New York, 1937. Pp. x + 1080. Price $7.50.

The author, who is now the Dean of the School of Chemistry and Physics of the Pennsylvania State College, has had a wide range of experience in general and organic chemistry. The present volume is the product of the author's experience in teaching and research. In the preface to his book, he says:

> The purpose and scope of this work can best be indicated by characterizing it as a one-volume "Beilstein" designed for practising organic chemists, for others who have to take occasional cognizance of organic compounds and their reactions, and for students who have pursued organic chemistry for at least a year with the aid of the many excellent elementary and intermediate textbooks now available. All efforts have been directed to making it a text of advanced character designed for those already possessing reasonable knowledge and experience in organic chemistry.

The book consists of four major parts, designated, respectively: Aliphatic Compounds; Alicyclic Compounds; Aromatic Compounds, and Heterocyclic Compounds. The following subjects are discussed in the several chapters: Part I—Hydrocarbons; Halides; Alcohols; Ethers; Sulfur Compounds; Esters of Inorganic Acids; Nitro and Nitroso Compounds; Amines and Related Compounds; Compounds of the Phosphorus Family; Metal Alkyls and Related Compounds; Aldehydes and Ketones; Monobasic Acids; Derivatives of Acids; Polyhydric Alcohols and Related Compounds; Hydroxyaldehydes and Hy-

droxyketones; Hydroxyacids; Dicarbonyl Compounds; Aldehyde and Ketone Acids; Polybasic Acids; Cyanogen and Related Compounds; Miscellaneous Compounds containing a Single Carbon Atom; Purines and Derivatives; Carbohydrates; Amino Acids; Proteins; Part II—General Discussion; Cyclopropane; Cyclobutane; Cyclopentane; Cyclohexane; Bicyclic Terpenes; Tricyclic Terpenes; Sesquiterpenes; Carotenoids; and Cholane Series; Part III—Benzene, its Homologs and their Derivatives; Polynuclear Hydrocarbons and Derivatives, and Naphthalene and other Condensed Ring Compounds; and Part IV—General Discussion; 5-Membered Rings; 6-Membered Rings, and Alkaloids.

The student and reader will find in Whitmore's *Organic Chemistry* a wealth of up-to-date information. He will find the subjects well arranged and ably discussed. The index is of more than ordinary value. It should find a welcome place in the reference collections of our colleges and universities and in technical libraries.

Colloid Chemistry, ed. 4. By JEROME ALEXANDER. D. Van Nostrand Company, Inc., New York, 1937. Pp. xviii + 505, figs. 43. Price $4.50.

This is the fourth edition of a book which has grown from modest beginnings. The author, who is a consulting chemist and chemical engineer, has worked fruitfully in a field of chemistry which is important in the fields of both theoretical and applied science. In the preface to the fourth edition, attention is directed to the fact that

> In preparing this greatly enlarged fourth edition, the author has adhered to the principles which governed the preparation of its predecessors: (1) assemblage of experimental data into naturally coordinated and interlocking groups, to form a broad mosaic which gives a coherent picture of nature; (2) breaking down the artificial mental barriers arising from scientific specialization and its incidental Babel of scientific jargon, so that the resources of many fields of investigation may be considered and both breadth and depth of mental focus acquired.

There are in the book 24 chapters, a bibliography, a glossary, and author and subject indexes. The designations of the several chapters follow: Historical and Introductory; Material Units and the Forces Dominating Them; A Simple Principle Underlying the Colloidal State; Classification of Colloids; Consequences of Subdivision; Optical Properties of Colloids—The Ultramicroscope; Determination of the Size and Mass of Colloidal Particles; General Properties of Colloids; Practical Applications of Colloid-Chemical Principles (8 chapters); Proteins and Carbohydrates; Biology and Medicine; Genetics; Hormones, Vitamins; Physiology and Pathology; Digestion; Bioelectricity; and Experimental Suggestions or Laboratory Manual.

Even the casual reader will be impressed by the wide field of interests covered by the present treatise. The theoretical considerations outlined by the author are followed by a discussion of colloidal chemistry in its relation to industries and agriculture. For instance, in chapter 9 the author refers to

applications in the fields of astronomy, meteorology, chemical warfare, geology, precious stones, etc. In chapter 10 he deals with applications in the wide field of agriculture. Similarly, reference will be found in other chapters, to the dye industry, the manufacture of soaps, the use of fuels, sewage disposal, photography, brewing, tanning, paper making, rubber manufacture, and a large number of other subjects.

The book lends itself to use as a text and as a source of reference material. It is undoubtedly a distinct addition to the now available treatises on colloid chemistry.

The Production of Field Crops, ed. 2. By T. B. HUTCHESON, T. K. WOLFE, and M. S. KIPPS. McGraw-Hill Book Company, Inc., New York and London, 1937. Pp. xvii + 445, figs. 110, tables 62.

This is the second edition of a book originally published in 1923. The authors indicate in the preface to the second edition that the favorable reception of the first edition has encouraged them to bring their material up to date. They note that "during the past thirteen years much new information on crop production has been contributed by the agronomists of the world. In fact, the contributions of new knowledge are so rapid that no textbook on this subject can ever be said to be truly up to date."

The book is divided into 9 sections, 41 chapters, and contains a satisfactory index. The first section represents a general discussion of the subject dealt with in the book. The following sections are devoted, respectively, to cereal or grain crops; legumes for seed; forage crops; root crops; fiber crops; tubers; sugar plants; and stimulants. The individual chapters are designated as follows: Beginnings of Plant Culture; Economics of Crop Production; Adaptation of Crops; Classification of Field Crops; Germination and Growth; Plant Improvement; The Value and Use of Good Seed; Commercial Fertilizers; Barnyard Manure; Lime; Preparation of the Seedbed; Seeding Practices; Tillage; Harvesting and Storage of Grain Crops; Haymaking; Silage; Pasture and Meadow Management; Weeds; Crop Rotation; Cereals; Corn; Wheat; Oats; Barley; Rye; Buckwheat and Rice; Peanuts; Soybeans; Cowpeas, Field Peas and Field Beans; Pasture and Hay Grasses; Clovers; Alfalfa; Sorghums; Millets, Vetches, Rape and Sunflowers; Sweet Potatoes; Carrots, Mangels and Turnips; Cotton; Flax and Hemp; Potatoes; Sugar Beets and Sugar Cane; and Tobacco.

It is obvious that the authors have taken into consideration all of the important staple crops. They have dealt with those grown in different regions of the United States. The more recently introduced economic plants, as well as those that have been cultivated in the United States since the early colonization of North America, are discussed in this book. Varieties, cultural methods, plant diseases, and insects are included in the discussion. The reader will find the book well arranged. Each chapter contains a list of appropriate references and suggested topics for discussion. As a general text on field crops, it should find a place in the classroom and in the reference library.

Weather Rambles. By W. J. HUMPHREYS. The Williams & Wilkins Company, Baltimore, Md., 1937. Pp. 265, illus. 33, charts 3. Price $2.50.

This attractive little volume offers much timely and interesting information. The author is not unaware of the poetry as well as of the practical meaning of weather. He tells us in his preface:

> Just as one enjoys roaming now and then over this or that part of the world, as whim or fancy may select, so too it is pleasant when the *wanderlust* is upon us, to ramble unconstrained hither and yon through the realm of science. And, like the Ancient Mariner, we just must tell our adventures to others. Such was the origin of "Weather Rambles," assembled here, some told for the first time, and some, by kind permission, retold with variations.

The contents of the book are divided into 22 chapters, designated as follows: Tall Tales of the Prairie Twister; Ice Ribbons; Gala Snow; The Murmur of the Forest and the Roar of the Mountain; Winter's Music; If Greenland's Ice Should Melt; Teetering on an Ice Age; Bread-and-Butter Meteorology; The Stuff We Call Air; Atmospheric Odds and Ends; Weighing and Counting the Air; How the Earth Got its Atmosphere; The Structure of the Atmosphere and Aviation; Sunshine Versus Starshine; Why the Higher the Colder; The Falling of the Dew; Frost Temperature; Where the Rain Comes from; A Noah's Flood a Day; Little Rain, Less Rain, Much Rain More Rain; Hunting Weather; and Home-made Weather.

The student, the business man, and the casual reader could well afford to spend a pleasant hour in reading this book and in gaining from it inspiration as well as solid information. In view of what the author has told us, we may feel tempted to qualify the statement once made by Mark Twain to the effect that everybody talks about the weather but nobody does anything about it.

Chemistry and Technology of Wines and Liquors. By KARL M. HERSTEIN and THOMAS C. GREGORY. D. Van Nostrand Company, Inc., New York, 1935. Pp. xxi + 360, tables 41, figs. 50. Price $5.50.

There is no doubt that in the fields of brewing, distilling, and wine-making the technician finds much to think about. The authors were well aware of this when they said in their preface:

> It is hoped that the present volume will, in a sense, serve to mark the end of an era, and the beginning of a new one. Mankind has had certain arts from time immemorial. Weaving, smelting, pottery, and the production of alcoholic beverages are noteworthy among these. And they share, besides great age, the distinction of having reached a fairly high peak of perfection without that intensive application of scientific development which has been characteristic of the newer arts whose origin has been in the advance of scientific knowledge.
>
> This is not to say that they have been untouched by science until the twentieth century. In particular the art of alcoholic beverage owes much to the workers of the nineteenth century. Pasteur, Hansen, Lavoisier, and many of the immortals of science have left their imprint and monuments in this field as well as in many others.

The book contains 16 chapters, designated as follows: Theoretical Considerations—Sugars and Starches; Theoretical Considerations—Enzymes:

Theoretical Considerations—Fermentation; Theoretical Considerations—Raw Materials; Yeasts and Other Organisms; Production of Yeast; Malt; Distillation; Whiskey Manufacture; Brandy, Rum, Gin, Applejack and Minor Distilled Liquors; Wines, Champagne and Cider; Liqueurs and Cordials; Analysis of Alcoholic Beverages—Interpretation; Analysis of Alcoholic Beverages—Methods; Analytical Reference Tables; Statistics of the Liquor Industry. It also contains selected bibliographies and an index.

The data offered by the authors will be of interest to the chemist and to the microbiologist, as well as to the technologist in a special field. Because of the use of large quantities of agricultural products as raw materials in brewing and distillation, the student of land use and soil management will also find in this book material of distinct interest.

Annals of Botany, New Series Vol. I No. 1, January, 1937. Edited by V. H. BLACKMAN. The Oxford University Press, Great Britain.

This volume contains a series of articles of interest to many workers in the general field of botany. Some of the articles will be of particular interest to the plant physiologist and to the soil scientist. The following papers, some of them very well illustrated, appear in this volume: "The Effect on the Behaviour of Stomata of Alternating Periods of Light and Darkness of Short Duration," by F. G. Gregory and H. L. Pearse; "Studies in the Inheritance of Physiological Characters III. Hybrid Vigour in Tomatoes Part I. Manifestations of Hybrid Vigour from Germination to the Onset of Flowering," by Eric Ashby; "Number and Behaviour of Chromosomes in Aloe litoralis," by T. K. Koshy; "Studies in the Pathogenicity of Tropical Fungi I. On the Types of Infection Encountered in the Storage of Certain Fruits," by R. E. D. Baker and C. W. Wardlaw; "The Morphology and Cytology of Typhula Trifolii Rostr.," by Mary Noble; "Contributions to the Study of Lachnea melaloma," by H. C. I. Gwynne-Vaughan; "The Development of the Vascular System in Evergreen Leaves More than One Year Old," by J. H. Elliott; "Chromosome Studies in Hyacinthus orientalis L. I. The Somatic Chromosome," by S. P. Naithani; "A Study of the Effect of Blue-Violet Rays on the Formation of Carbohydrates in Leaves," By R. H. Dastur and S. Solomon; "The Influence of Environment on the Growth and Metabolism of the Tomato Plant II. The Relationship between Water Content and Assimilation," By R. Melville; "The Effect of Alternate Periods of Light and Darkness of Short Duration on the Growth of the Cucumber," by G. B. Portsmouth; and "The Interaction of Factors in the Growth of Lemna X. The Interaction of Nitrogen and Light Intensity in Relation to Respiration," by H. L. White and W. G. Templeman.

Sheep Farming. By ALLAN FRASER. Crosby Lockwood & Son Ltd., London, 1937. Pp. 178, illus. 11. Price 7/6.

The author is Deputy Director for Research of Duthie Experimental

Stock Farm of Rowett Institute, Aberdeen, Scotland. The Director of the Institute, Sir John Orr, has written a foreword. Sir John's statement may be quoted for the benefit of the readers. He says:

> Whatever changes may be brought about in British agriculture by mechanization and Government marketing schemes and other measures applied with the intention of promoting its welfare, sheep farming must always retain an important place in our system of agriculture. There are large areas, especially in the West of Scotland, the North of England, and in Wales where the land is unsuitable for anything except sheep farming. In the grain-growing districts of the Eastern Countries, the benefit of even manuring and consolidation which certain types of corn land derive from sheep, makes feeding of sheep worth while, apart from the profit on the sheep themselves. Indeed, there are very few farms where either a permanent or a temporary flock of sheep does not fit into the general economy for the profitable utilization of pastures.

The 19 chapters of which the book is made up are designated as follows: The Sheep Industry To-day; Sheep Breeds; Controlling Fertility; Wintering Ewes; Preparing for Lambing; Lambing; The Young Lambs; Rearing Lambs; Pastures; Sheep-Feeding; Summer Tasks; Buying Sheep; Selling Sheep; Controlling Disease; Sheep Vermin; Sheep Worms; Sheep Diseases—Ewes; Sheep Diseases—Lambs; Sheep Future.

The student of agriculture in general, and of livestock husbandry in particular, is well aware of the fact that sheep raising is more prominent in new agricultural regions than it is in the older regions. Our census records show clearly that in the United States sheep raising has all but disappeared in the Northeast and has declined greatly in the Middle West and other agricultural areas. There is something impressive in the history of sheep raising in Western Europe. A striking decline has occurred in the number of sheep in most of the European countries. There has been a decrease in numbers even in the British Isles. It is remarkable, none the less, that, in a relative sense, sheep raising has held its own in England, Scotland, and Wales.

Some of the newer knowledge concerning grassland management and animal breeding and nutrition leads one to recognize that sheep raising may anticipate a revival in many places. Certainly, the Rowett Institute has been one of the notable contributors to our newer knowledge of livestock management. The present volume, well printed and attractively illustrated, is a most acceptable addition to our knowledge of animal husbandry.

Sod House Days Letters from a Kansas Homesteader 1877–78. Edited by John Ise. Columbia University Press, New York, 1937. Pp. xii + 148. Price $2.75.

The Columbia University Press is engaged in the task of resurrecting for the thoughtful person the experiences, the observations, and the outlook of the pioneers in North America. In the editor's foreword, we find the following statement:

Probably no single factor has so profoundly affected American development and shaped American ideals as the West and its frontier. Its influence upon our agriculture, industry, politics, education and literature—in fact upon every phase of institutional life—can scarcely be overestimated.

The conquest and settlement of the West by the white man was already well under way during the Colonial period. Indeed, long before the outbreak of the Revolution the mighty movement destined to carry courageous settles over the slopes of the Alleghenies into the alluvial valley of the Mississippi, out across the plains, and ultimately to the waters of the Pacific was already under way.

Elsewhere he says:

But most numerous of all were those who turned to the West, with its abundance of free or relatively cheap lands, in search of economic betterment. To the struggling Eastern farmer, dissatisfied tradesman, religious dissenter, oppressed mechanic, or ambitious young lawyer, or the zealous missionary seeking fresh spiritual conquests, the West was a sort of Promised Land, the gates of which were ever open. . . . This volume records the experiences of one of that great army of settlers from the East and from the Old World who crowded westward during the eighteen-seventies, just after the heyday of Indian fighting and buffalo hunting. The book is doubly significant. Not only does it portray the hardships of pioneering, but it also furnishes firsthand information about the status of agriculture in a west central Kansas community, at a time when the seeds of agrarian discontent were being widely sown.

The reader will find in this book a most fascinating story that reflects the experience of many. It portrays something of the evolution of American agriculture, of the struggles of pioneers in an unfriendly environment, and of individual and community achievements that have become a part of our national history. The reader will find himself well repaid in acquainting himself with the contents of this book.

The Nature and Properties of Soils, ed. 3. By T. Lyttleton Lyon and Harry O. Buckman. The Macmillan Company, New York, 1937. Pp. xiii + 392, figs. 45, tables 42. Price $3.50.

This is the third edition of a book well known to many teachers of agriculture and to many students of soils and of agronomy. The purpose of the authors in preparing this book is indicated in the preface. They say:

This volume is designed for the student who is interested in the nature and properties of soils and their relationships to higher plants. A working knowledge of inorganic chemistry is presupposed as well as some understanding of the colloidal state of matter. A background of general geology and biology will be exceedingly helpful.

The book contains 17 chapters and author and subject indexes. The several chapters are designated as follows: A Fundamental Concept of the Soil; The Supply and Availability of Plant Nutrients in Mineral Soils; The Physical Properties of Mineral Soils; Colloidal Clay and Ionic Exchange; The Organisms of the Soil; The Organic Matters of Mineral Soils: Forms of Soil-Water and

their Plant Relationships; Soil-Moisture Losses and their Control; The Origin and Classification of Soil Materials; Soil Formation, Classification, and Survey; The Nature and Utilization of Organic Soils; The Soil Reaction—Soil Acidity and Alkalinity; Liming the Soil; The Nitrogen Economy of Soils; Fertilizers and Fertilizer Practice; Farm-Manure and Green-Manure; and The Methods of Fertility Maintenance for Mineral Soils.

The present edition not only reflects the familiarity of the authors with their subject, but also represents an effort to create a balanced treatise on the general subject of soils. The remarkable expansion of our knowledge in the fields of agriculture, geology, climatology, soil physics, soil chemistry, soil microbiology, and plant physiology makes it difficult for any author to write a book that would, without being too bulky, include all of the significant facts relating to soils and soil fertility. The authors have been fortunate in dealing with their subject in such a manner as to have produced a very satisfactory textbook. The teacher of soils and the agronomist will find themselves under added obligation to the authors.

The World of Atoms, ed. 2 By ARTHUR HAAS. D. Van Nostrand Company, Inc. New York, 1937. Pp. xiv, + 183, figs. 54, tables 6.

This is the second edition of a book whose author is Professor of Physics at the University of Notre Dame. It was translated by George B. Welch of Northeastern University. The translation of the first edition of the same book was made by Horace S. Uhler, Associate Professor of Physics at Yale University.

To quote from the preface to the second edition:

> In the eight years that have elapsed since the appearance of the first edition of this book few achievements have aroused such widespread interest as the revolutionary discoveries in atomic physics. New building-stones of matter have been revealed; trustworthy experiments have demonstrated the production and annihilation of matter; and in such common substances as water important and unsuspected admixtures have been found. Most extensive progress has been made in the disintegration and transmutation of the chemical elements and in the production of radioactivity; today, by a profusion of astonishing experiments, old ideas of alchemists in modern form and on an exact foundation have become realities. There is in the process of development a new science of atomic nuclei which perhaps conceals within itself unsuspected possibilities of future industrial exploitation.

The 12 lectures of which this book consists are designated, respectively: Matter and Electricity; The Building-Stones of Atoms; Light-Quanta; Spectra and Energy Levels; The Elements; The Atom as a Planetary System; Molecules; Radioactivity; Methods of Atomatic Disintegration; The Results of Atomic Disintegration; Cosmic Rays, the Positron, and Induced Radioactivity, and Wave Mechanics of the Atom. There are also a name index and a subject index.

The general reader, as well as the student of physics and chemistry, should

find in this book much of interest presented in readable form. The clarity of the discussion is helped by the illustrations contained in the book.

Biochemistry Applied to Malting and Brewing. By R. H. HOPKINS AND B. KRAUSE. D. Van Nostrand Company, Inc., New York, 1937. Pp. 342, figs. 5. Price $4.75.

The senior author is Professor of Brewing and Industrial Fermentations in the British School of Malting and Brewing at the University of Birmingham. The junior author is Head Chemist of the Tuborg Breweries in Copenhagen, Denmark, and Lecturer at the Scandinavian Brewing High School in Copenhagen.

The authors state in the preface that

> The need for textbooks in English dealing with the scientific principles and technology of malting and brewing must have been felt by many engaged in the industry for some years past. This book is meant to supply a part of the need. It is not a textbook of brewing, there being no description of plant or of materials used for its construction, and a minimum of technology. Indeed, a general knowledge of the technology of malting and brewing, either the decoction or the infusions systems, either the bottom or top fermentation, systems is assumed.

Elsewhere they say:

> The main part of the book, Chapters III, IV and V, treats of the applications of modern biochemistry to the technology of malting, brewing, and fermentation respectively. One purpose here has been to indicate the influences of varied extraneous conditions, another to enable the practical maltster or brewer to draw well founded conclusions from analytical results of barley, malt, hops, wort, etc.

There are five chapters in the book, designated, respectively, as: A Review of Some Important Principles of Physical Chemistry; General Chemistry of the Raw Materials of Malting and Brewing; Malting; The Brewing Processes, Mashing, Boiling, Cooling, and Fermentation; and The Finished Beer. There are also a list of references, an appendix, and an index.

The applied field of the general subject dealt with has been well explored by the authors and is effectively presented for the benefit of the reader and student.

Man in a Chemical World. By A. CRESSY MORRISON. Charles Scribner's Sons, New York and London, 1937. Pp. xi + 292, illus. 11.

To the man in the street, chemistry is a world of mystery. Intelligent persons are trying to interpret their lives and activities with the help of the newer knowledge that chemistry has given us. In the foreword to the work, written by Prof. Arthur W. Hixson, of Columbia University, we find the following:

> This is the book. It is a proud record of the accomplishments of man in the rôle of creator. It was not intended that it should be a profound contribution to the literature of

theoretical or applied chemistry. However, for the person who will take the trouble to read it, there will be a full measure of enjoyment and a broadening general knowledge of his relationship to material things. He will have a better understanding of the part that applied chemical science has had in raising the plane of his living to a higher level than that enjoyed by any previous generation. The authorship has been in good hands. Seldom does one find a writer who can present the accomplishment of a great industry with such enthusiasm and charm. This book is the clear sparkling distillate of the wide experience gained during a lifetime of devoted service to an industry that has helped mightily to strengthen the pulse of the nation from its very beginning.

The topics dealt with by the author are designated as follows: Nature Points the Way; Chemistry in Overalls; Keeping Well; Feeding Millions; Wheels and Wings; From Papyrus to Television; All the Comforts of Home; Serving Industry; Security; The More Abundant Life; The Crystal Reveals; and L'Envoi. There is also an index. The book is well printed, is attractively illustrated, and is certain to supply enjoyment as well as information to the reader.

JACOB G. LYMAN.

THE RECLAMATION OF THE DUTCH SALINE SOILS (SOLONCHAK) AND THEIR FURTHER WEATHERING UNDER THE HUMID CLIMATIC CONDITIONS OF HOLLAND[1]

D. J. HISSINK

Institute of Soil Science, Groningen, Holland

Received for publication August 6, 1937

MUD DEPOSITS, KWELDERSOILS

The Dutch saline soils occur on the seacoast. The sea deposits are brought in by the recurrent tides against the sea dikes. They vary from very sandy to heavy clayey soils; the reclamation of the latter only is discussed here.

The sea deposits contain the salts of the sea water, and of the exchangeable bases, the MgO and the Na_2O are more prominent than the CaO. These deposits contain approximately[2] 25–40 parts CaO, 45–30 parts MgO, 8 parts K_2O, and 20 parts Na_2O per 100 parts exchangeable bases and are therefore to be regarded as magnesium-sodium clay soils. The chief feature of these saline clay deposits is their muddy structure, or rather their lack of structure, which is directly related to the very high content of water. Whereas the normal older clay soils in the wettest condition contain no more than about 50 gm. H_2O per 100 gm. clay substance,[3] the mud deposited by the sea water against the dikes contains about 175 to 350 gm. H_2O per 100 gm. clay-substance. This mud is characterized by its dark color, due to the presence of ferrosulfide (FeS), which has been formed from the $CaSO_4$ of the sea water and the Fe_2O_3 of the soil. When in contact with air, the FeS oxidizes to $FeSO_4$, which is immediately converted by the superabundant $CaCO_3$ into $CaSO_4$ and $FeCO_3$, and the $FeCO_3$ then oxidizes to Fe_2O_3. The dark color changes during this process to a gray tint.

Because of continuous accretion, the mud deposits gradually accumulate, until they are too high to be covered by the normal summer tides. It is obvious that the reclamation of these Dutch saline soils has, in the humid Dutch

[1] A few papers on this subject have already appeared (10, 12, 13, 14, 16), some in English, some in German, and some in Dutch, which are not readily accessible to most American readers. After discussing the matter, Doctor Lipman and I came to the conclusion that it might be useful to publish this paper in SOIL SCIENCE.

[2] The method for the determination of the exchangeable bases in saline soils which also contain calcium carbonate has not yet been accurately fixed. I have endeavored to indicate an approximate method (9, 11), but I am quite willing to admit that there is much to criticize in this method, by which the given figures have been obtained.

[3] By "clay-substance" is understood fraction I + II, that is, particles smaller than 16μ in diameter. The settling velocity of these particles is 10 cm./450 seconds. According to Stokes' formula $V = 34720\, r^2$, therefore, the diameter ($2r$) = 0.0016 cm. = 16μ.

climate, a somewhat different aspect from that of similar reclamation in semi-arid and arid regions, such as those of Hungary, southern Russia, and California. The annual rainfall in the Netherlands is sufficient entirely to wash out the salts within a few years, provided the drainage conditions are otherwise good. When the muddy deposits have accumulated sufficiently to rise above the normal summer tides, the salts are consequently washed out of the upper 20 to 30 cm. of the muddy deposits by the summer rains to such an extent that the original salt vegetation (Seaweed, Salicornia, etc.) gives way to a grass flora. These grass-grown deposits are called "kwelders"; they are covered only by the high tides, especially in winter. When the kwelder is high enough a dike is built to keep out the sea water; the kwelder is now transformed into a young sea-polder.

The muddy deposits are therefore to be regarded as the first step in the formation of marine clay soils, which are well known to be the most fertile soils in the Netherlands, both from a chemical and from a physical point of view. For many decades the young marine clay soils yield abundant crops, without the use of any fertilizer, and even at a great age (200 to 400 years) they are regarded as among the most valuable arable lands. It is therefore obvious that the study of the transition from muddy deposits to fertile land and of the further weathering of the young marine clay soils is worthy of the interest of Dutch soil scientists.

RECLAMATION OF THE SALINE MUD DEPOSITS

The first soil-forming process of the saline mud deposits is that of drying, which takes place as early as the kwelder period, thus while the land is not yet diked but already bears a grass flora. It is obvious that this drying process takes place from the upper layer, because of the action of the sun and the wind. Gradually the deeper layers—also under the influence of the grass flora—also dry up.

As a result of the high content of water the mud deposits have a very low volume-weight [weight of 1 cc. dry matter (105°C.) in grams]. The volume-weight of the mud deposits was found to range from 0.43 to 0.81, depending on the water content; that of the upper layer of the kwelder soil was about 0.87; that of the upper layer of the 6-year-old new polder soil was about 0.97; and that of the oldest polder soils was 1.35. During the drying process, therefore, a large number of fissures are formed, at first in the upper layer. The muddy mass, which at first is virtually impermeable to water, gradually becomes permeable and attains solidity, or structure.

After the formation of fissures the rain water is able to penetrate into the soil and to wash out the salts of the sea water, at first, it is true, only out of the upper layer. The air, too, penetrates into this upper layer. Oxidation processes take place; the sulfur-iron compounds are converted into iron oxide (Fe_2O_3) and sulfuric acid (H_2SO_4). As $CaCO_3$ is present, gypsum ($CaSO_4$) and calcium bicarbonate, two calcium salts soluble in water, are

formed, and these change the sodium-magnesium-clay-humus substance into calcium-clay-humus substance. This part of the soil-forming process will be treated in detail below.

For the rapid course of this exchange process it is of importance that the exchange products, and especially the sodium salts, should be removed from the soil as quickly as possible. A thorough and rapid draining of the rain water from the upper layer into the ditches and canals is therefore necessary.

It is of great importance that the soil become permeable to water before the end of the salt period, that is, while the soil still contains salts which prevent the peptization of the sodium-clay-humus substance. If the soil has become sufficiently permeable to water before the end of the salt period, the salts, and later the products of the exchange process also, and especially the sodium salts, can be rapidly carried off. The peptization of the clay-humus substance, which will unavoidably occur after the washing out of the salts, naturally somewhat reduces the permeability of the soil for a time. The already fairly permeable soil can, however, afford this luxury. If, on the other hand, the washing out of the salts from the soil occurs in the muddy period, the soil may, because of the peptization, become almost impermeable to water.

It is a fortunate circumstance that the soil—at any rate, that in the upper layer—dries somewhat during the kwelder period, its structure becoming such that the soil is more or less permeable to some depth, and at the same time the base equilibrium shifts from the Na side in the Ca direction. Thereby the soft skeleton of the young sea-soil is somewhat solidified.

For the formation of a good structure it is therefore essential that two processes take place, one physical, the other chemical. The muddy mass must dry, and the exchangeable sodium (and magnesium) must be largely replaced by calcium. Neither of these processes is in itself sufficient for the formation of a good structure. Both the sodium-clay substance and the sodium-humus substance disintegrate again or become soft when, after drying, they again come into contact with water. And as long as the calcium-clay-humus substance remains more or less soft, it does not form a good material for a strong soil-skeleton. According to various investigators [among others, Sokolovsky (19)] a difference occurs in this respect between the clay and the humus substance. It is claimed that the calcium clay, after drying, remains a fully reversible colloid; the dried calcium humus, however, is a partly irreversible colloid. The dried irreversible humus does not peptize, even when part of the lime is washed out.

In this connection it is of importance to note the possibility of a rather close connection between the organic and the inorganic adsorbing complexes. According to Gedroiz (4) and others (1) it is possible that in soils these two parts form, not a mechanical mixture, but something more intimate. Humus, as a colloid of high dispersivity, binds the soil particles together, even when there is but a small percentage of it.

In the structure-forming process of the muddy deposits it is therefore es-

sential that both the clay and the humus substance dry and the excess of exchangeable sodium (and magnesium) be replaced by calcium. I am convinced, although I cannot prove by submitting figures, that the old grass flora in the kwelder period has a very great influence on the formation and strengthening of the structural solidity of the young kwelder soil. From the soil science point of view the kwelder period is to be considered as an extremely useful preliminary period for the future polder soil. During this kwelder period the foundation is laid for the excellent structure of our marine clay-polder soils, a structure which remains unchanged for centuries. Williams (3, 20) long ago pointed out the great importance of a durable structure in all soils for all soil processes. On the basis of his studies of the natural soil-formation processes, Williams considers that structure in soil is the product of meadow formation and results from the anaerobic decomposition of organic residues, and that the creation of durable structure in arable soils can be accomplished only by meadow-forming plants, that is, in the turf stage of soil formation. Since newly formed structure is inevitably lost, Williams advises that arable soil be periodically subjected to the influence of perennial meadow plants as a part of the process of cultivation.

After a sufficiently long kwelder-period the young polder, immediately after being diked, can be cultivated as arable land. With proper drainage the salts are washed out in one winter to a sufficient depth. The chief requisite in the cultivation of the young polder soils is that the structure formed during the kwelder period should not be destroyed. The main point here is that the working of the soil should at first be only very superficial and that it should not go any deeper than the drying process, that is, than the depth to which the formation of structure has progressed. More especially the soil should not be worked in a wet condition. Care must further be taken that the water be rapidly led away; pools of water must not occur. It must constantly be borne in mind that this is still a young soil, which is more rapidly peptized by water than is an old soil.

As the young polder soil ages, drying—with the consequent formation of fissures, to which must be added worm and root holes—gradually occurs to a depth of more than 1 meter. During this process, soils of extraordinarily great permeability are formed to a great depth. As a result of cultivation, the upper layer gradually becomes less permeable; under the upper layer in many places is a thin layer which is fairly impermeable to water (Pflugsohle). In soil profiles of this nature the rain water will take the shortest way through the fairly permeable upper layer (0 to about 20 cm.); the movement of water will here be directed almost vertically downward. In the very permeable lower layer (from 20 to 100 or 150 cm.) the rain water has a chance to flow away very rapidly to the open ditches or to the drainpipes.

I have been able to observe this great permeability of the lower layers very well on a plot in a polder 163 years old. The plot is 43.5 m. wide and is drained by two side ditches about 1.5 to 2 m. deep; further drainage by

means of open ditches or drains is not provided. Nevertheless this piece of ground never suffers from an excess of water.

As the soil ages still further the permeability of the deeper layer gradually decreases, and consequently drainage becomes necessary.

RECLAMATION OF THE NEW ZUYDER ZEE SOILS

A dam, 30 km. long, has been built from the coast of the province of Noord-Holland, by way of the island of Wieringen, to the coast of the province of Friesland. In 1932, when this dam was completed, the Zuyder Zee was transformed into a lake with an area of about 335,000 hectares. It is now intended to dike within this lake four polders, of respectively 20,000, 55,000, 95,000, and 55,000 hectares, an area equal to 7 per cent of the total area of Holland and to 10 per cent of the area now available for cultivation. In 1930 a dike from Medemblik to the island of Wieringen was completed, enclosing an area of about 20,000 hectares. The water was pumped out of this enclosed area, this process being finished in September, 1930; the new polder is known as the "Wieringermeer polder." The new soils vary from very sandy to heavy clay soils; the reclamation of the latter only is discussed here.

Immediately after being freed from the sea water these soils presented the same picture as do the muddy deposits when these are formed on our coasts: a muddy mass, virtually without structure, very rich in salt water and free from any vegetation. These new soils were very quickly covered with the usual salt flora (seaweed, salicornia, sea aster, etc.).

For these soils, too, the first soil formation process to be undergone is that of drying. They have to get rid of their surplus water, and so dry, form fissures, and become permeable to water; in a word, as a result of this process of drying, the originally structureless soil is changed into one with structure. For the carrying-off of the surplus water, canals and wide ditches have to be dug, and the water is removed from the smaller plots by means of open ditches and drains.

It would be best to leave these new Zuyder Zee soils for a decade or two as meadowland; in other words, to let them, like the muddy deposits along our coasts, remain for a sufficiently long time in the kwelder stage. This is necessary to give firmness to the mineral and organic soil colloids and thus to render the soils more capable of resisting the peptizing action of the rain water. As long as sodium comprises 15 to 20 per cent of the exchangeable bases, the risk of peptization is very great, and even after this sodium is nearly washed out and replaced by CaO, the new soil for a time remains liable to peptization by water.

RECLAMATION OF THE FLOODED OLDER POLDER SOILS

From time to time a sea dike gives way, and the polders behind it are flooded with the salt sea water, with which they then remain for some time in contact. After the dike has been repaired and the sea water has been ex-

pelled, a soil saturated with salt water is left behind, and moreover part of the exchangeable calcium of the soil has been replaced by sodium from the sea water. The salts of the sea water are rather quickly washed out, but the conversion of the sodium clay into calcium clay may sometimes take several years longer. The older polder soils differ chiefly in two respects from the young kwelder soils. First, the upper layer of the older polder soils is already well packed and has a high volume-weight. When drying takes place, fewer fissures are formed, and consequently the upper layer remains for a long time fairly impermeable to water and therefore also to air. This latter factor also results in a diminished production of carbonic acid, so that little calcium bicarbonate is formed. Secondly, the older polder soils lack sulfur-iron compounds. No $CaSO_4$, therefore, is formed, and the conversion of the sodium clay into calcium clay depends entirely on the calcium bicarbonate.

The best remedy for the sick older polder soils is to leave them as grassland for several years. More than a hundred years ago this procedure was recommended and applied by practical agriculturists (18).

TRANSFORMATION OF THE MAGNESIUM-SODIUM CLAY SOILS INTO CALCIUM CLAY SOILS

Both the deposits on our coasts and the new Zuyder Zee soils are rich in $CaCO_3$. When air penetrates into the soil, the FeS oxidizes into $FeSO_4$, which with $CaCO_3$ forms $CaSO_4$, and calcium bicarbonate is also formed. These two salts are soluble in water and convert the magnesium-sodium clay into normal calcium clay.

The rapidity with which this process takes place can be seen from tables 1, 2, and 3. These tables give the figures for soils taken from the following four polders: the experimental polder near Andijk, diked in 1927; the Carel Coenraad polder, the youngest polder in the Dollard region on the east coast of the province of Groningen, diked in 1924; the Reiderwolder polder, the youngest but one in the Dollard region, diked in 1862; and the Wieringermeer polder, diked in 1930.

The course of the transformation can easily be followed. The greater part of the exchangeable sodium is very quickly washed out and replaced by CaO from the $CaCO_3$. There is some difference between soils 5 and 6, both taken from the upper layer of the Andijk polder and both taken at the same time, viz., 8 years after diking. Soil 5, which is taken from a plot favorably situated with regard to drainage, has a relative proportion of $77 + 18 + 4 + 1 = 100$ (see table 3), whereas the relative proportion of soil 6, which is unfavorably situated in that respect, is of $61 + 29 + 5 + 5$. The exchangeable magnesium is much more strongly bound by the clay-humus substance (5); consequently even the soil of the most favorably situated plots contains 17 to 18 per cent MgO.

As is seen by soil 8 (table 3), the magnesium content of the 70 year old Dollard soil from the Reiderwolder polder has been reduced to the minimum

TABLE 1

Origin and age of soil

SOIL NO.	ORIGIN AND FURTHER PARTICULARS	AGE OF SOIL FROM DATE OF DIKING
		years
1	Andijk; the original black mud	0
2	Carel Coenraad polder; the layer from 40 to 50 cm. beneath surface, just free from water-soluble chlorides and sulfates; taken in 1930	6
3	The upper layer (0–14 cm.) at the same spot as no. 2, taken at the same time	6
4	Andijk; the upper layer of a plot situated very favorably in respect of the draining-off of water; taken in 1933	6
5	Andijk; the same plot as no. 4; upper layer (from 0 to between 10 and 33 cm.); taken in 1935	8
6	Andijk; the upper layer of a plot situated very unfavorably in respect of the draining-off of water; taken in 1935	8
7	Wieringermeer polder; average of several light clay soils, upper layer (0–10 cm.); taken in 1935	5
8	Reiderwolder polder; upper layer; taken in 1932	70

TABLE 2

*Content on $CaCO_3$, humus, clay, and sand; values S, V, and pH**

SOIL NO.†	PERCENTAGES CALCULATED ON DRY MATTER (105°C.)						*S* PER 100 GM. CLAY (+HUMUS)	*V*	pH
	$CaCO_3$	Humus	Clay	Sand	Clay (+ humus)	*S*			
1	11.6	5.7	63.1	17.5	89.0	27.4	30.8	....	
2	10.0	3.8	66.1	20.1	83.4	31.5	37.8	43.4	8.3
3	8.7	5.5	56.4	29.4	81.4	29.3	36.0	42.3	7.7
4	11.5	4.6	50.4	33.5	71.3	26.2	36.7	....	
5	10.8	5.2	57.2	26.8	80.8	30.0	37.1	....	
6	8.2	4.0	54.4	33.4	72.6	26.9	37.1	....	
7	11.0	1.7	17.4	69.9	25.1	9.85	39.3	....	
8	8.1	3.5	66.8	21.6	82.7	30.4	36.8	45.4	7.7

* Clay = fraction I + II, particles smaller than 0.016 mm. (settling velocity 10 cm./450 seconds); sand = particles from 0.016–2 mm. Clay (+ humus) is calculated by multiplying the humus by 4.545 and adding this to the clay; in this calculation the base-adsorbings capacity of the humus substance has been estimated as being 4.545 time greater than that of the clay substance. *S* value = sum of exchangeable bases ($CaO + MgO + K_2O + Na_2O$) in milligram equivalents; *V* value = the degree of saturation according to Hissink (baryta method). The pH value is determined in water suspensions by the quinhydrone method.

† See table 1.

value (relative proportion 86 + 9 + 4 + 1 = 100). How long is required for this replacement of exchangeable MgO by CaO under Dutch climatic conditions is unknown and will be determined during further investigation of the young polders.

FURTHER WEATHERING OF THE CALCIUM CLAY SOIL

After several decades the Dutch salt clay soil thus becomes a normal calcium clay soil, rich in $CaCO_3$ and with a relative base proportion of about 86 + 9 + 4 + 1 = 100. In the course of centuries this soil undergoes great changes. As with all investigations, so, also, in this case it is of great importance to find a good object of study. So far as the investigation of the process of weathering of the Dutch sea-clay deposits is concerned, the soils of the successively diked Dollard polders (Dollard region on the east coast of the province of Groningen) provide an ideal object of study. These polders were

TABLE 3

Exchangeable bases, relative proportion

SOIL NO.*	EXCHANGEABLE BASES IN MILLIGRAM EQUIVALENTS PER 100 CM. CLAY (+ HUMUS)					RELATIVE PROPORTION OF EXCHANGEABLE BASES PRESENT PER 100 PARTS EXCHANGEABLE BASES			
	CaO	MgO	K_2O	Na_2O	*S*	CaO	MgO	K_2O	Na_2O
1	7.3	14.9	2.6	6.0	30.8	24	48	9	19
2	15.9	11.3	2.6	8.0	37.8	42	30	7	21
3	23.8	9.2	2.3	0.7	36.0	66	26	6	2
4	27.9	6.3	1.6	0.9	36.7	76	17	5	2
5	28.6	6.6	1.5	0.4	37.1	77	18	4	1
6	22.7	10.6	2.0	1.8	37.1	61	29	5	5
7	28.6	6.7	1.9	2.1	39.3	73	17	5	5
8	31.6	3.3	1.5	0.4	36.8	86	9	4	1

* See table 1.

diked after the formation of the Dollard in the year 1277; the oldest polder dates approximately from the year 1550, and the youngest Dollard polder—the Carel Coenraad polder—was diked in the year 1924. The soil of the entire complex of Dollard polders is of a fairly homogeneous composition, consisting of very heavy clay soil containing little humus. Weathering has proceeded far enough to a fairly great depth. All stages are found, from the young soils, well-saturated with bases and rich in $CaCO_3$, of the young polders, to the very old soils, in which not only the calcium carbonate has disappeared, but in which the adsorbing clay-humus complex has also already lost a considerable amount of its bases, so that the soil now has an acid reaction. The soils of this latter stage are nearly 400 years old.

In this paper only a summary of a few results of the investigation of the surface of soils (from 0 to 20 or 25 cm.) of the Dollard polders is given (tables 4, 5, and 6).

TABLE 4

Origin and age of the soils of the Dollard polders

SOIL NO.	SOIL SAMPLE	NAME OF POLDER	YEAR IN WHICH THE POLDER WAS DIKED	AGE OF POLDER IN THE YEAR IN WHICH SAMPLES WERE TAKEN (1930, 1932, OR 1933)
				years
1	3648	Carel Coenraad polder	1924	6
2	5546/47	Reiderwolder polder	1862	70
3	5268/69	Finsterwolder polder	1819	113
4	5560/61	Oostwolderpolder	1769	163
5	5574/75	Het Nieuwland	1701	231
6	6181/82	Het Oud Nieuwland	1665	268
7	5588/89	Het Oudland	1626	306
8	3423	Polder Simson	1550	380

TABLE 5

Content on $CaCO_3$, humus, clay, and sand; values S, V, and pH—Dollard polders

SOIL NO.*	PERCENTAGES CALCULATED ON DRY MATTER (105°C.)						*S* PER 100 GM. CLAY (+ HUMUS)	*V*	pH
	$CaCO_3$	Humus	Clay	Sand	Clay (+humus)	*S*			
1	9.4	4.8	57.5	28.3	79.3	30.1	37.9	42.3	7.8
2	9.3	3.3	68.9	18.5	83.9	31.3	37.3	45.7	7.7
3	7.5	3.4	71.9	17.2	87.4	32.2	36.9	46.6	7.7
4	6.9	3.3	68.8	21.0	83.8	30.5	36.4	45.2	7.7
5	3.0	3.6	76.8	16.6	93.2	35.8	38.4	46.8	7.7
6	0.2	3.6	70.6	25.6	87.0	33.0	37.9	44.5	7.2
7	0	3.6	71.4	25.0	87.8	34.0	38.7	45.3	7.4
8	0	4.8	68.4	26.8	90.2	27.1	30.0	32.1	5.9

* See table 4.

TABLE 6

Exchangeable bases, relative proportion—Dollard polders

SOIL NO.*	EXCHANGEABLE BASES IN MILLIGRAM EQUIVALENTS PER 100 GM. CLAY (+HUMUS)					RELATIVE PROPORTION OF EXCHANGEABLE BASES PRESENT PER 100 PARTS EXCHANGEABLE BASES			
	CaO	MgO	K_2O	Na_2O	*S*	CaO	MgO	K_2O	Na_2O
1	22.5	10.5	2.2	2.7	37.9	59	28	6	7
2	31.7	3.9	1.4	0.3	37.3	85	10	4	1
3	32.9	3.0	0.9	0.1	36.9	89	8	2.5	0.5
4	31.6	3.1	1.5	0.2	36.4	87	8	4	1
5	34.0	2.9	0.9	0.6	38.4	88	8	2	2
6	30.3	6.9	0.6	0.1	37.9	80	18	1.5	0.5
7	31.3	6.3	0.7	0.4	38.7	81	16	2	1
8	21.2	7.3	0.6	0.9	30.0	71	24	2	3

* See table 4.

As van Bemmelen has already pointed out, the content of $CaCO_3$ decreases with the age of the soil; van Bemmelen found that approximately 1 per cent $CaCO_3$ was washed out from the soils of the Dollard polders in about 25 years (2). The humus content of the kwelder soil, when the latter is grown with grass, is 5.4 per cent. After diking, the pasture is converted into arable land, and the humus content diminishes to about 3.5 per cent, after which it remains practically constant. I may add here that at the same time the nitrogen percentage of the humus rises from about 5.4 to about 6 or 6.5, and that the P_2O_5 content is rather high, about 0.2 per cent, but decreases in the older polders (0.17–0.13 per cent).

From tables 4, 5, and 6 we see that the upper layer of the young normal marine heavy clay soils of the Dollard region, from perhaps a few decades after their diking, are characterized by the following figures: a humus content of about 3–3½ per cent, or 5 gm. humus per 100 gm. clay substance; an *S* value of about 37–38 m.e. per 100 gm. clay (+ humus); a *V* value (according to Hissink) of about 42–47; and a pH of about 7.7; and the average relative proportion of the exchangeable bases is 87 + 8 + 4 + 1 = 100. At first (soil 2, Reiderwolder polder) the $CaCO_3$ content is 9–10 per cent (9.3 per cent). As is well known, the $CaCO_3$ decreases until, after about 250 years, it is washed out of the upper layer. As long as the soil still contains $CaCO_3$, the values *S*, *V*, pH, and the relative proportion remain nearly constant, the average values in this period, from about 50–250 years, being *S*(per 100 gm. clay + humus) = 36.9, *V* = 45.8, pH 7.8 to 7.7, and the relative proportion 87 + 8 + 4 + 1 = 100. A consideration of the figures of the following polders (soil 6, 268 years old, and soil 7, 306 years old) shows that something very remarkable takes place. The $CaCO_3$ is entirely or virtually washed out of the upper layers of these polders. As I had anticipated, the content of exchangeable CaO decreases in this period, but the remarkable thing is that this loss of CaO is compensated for by a rise of the content of exchangeable MgO, with the result that the values, *S*, *V*, and pH remain practically unchanged in this period. In the upper layer of the 306-year-old polder, soil 7, that is, about 50 years after the disappearance of the $CaCO_3$, *S* is still = 38.7, *V* = 45.3, and pH = 7.4. The exchangeable MgO increases, however, so that the average relative proportion in this stage becomes 81 + 16 + 2 + 1 = 100. In this stage of the weathering process the exchangeable CaO washed out seems to be replaced by MgO. It is necessary to inquire where this exchangeable MgO comes from. In my paper of 1920 (6, 7), I pointed out the possibility of the change of the bases from the acid-soluble form into the exchangeable form. In that paper I also pointed out that among the acid-soluble bases of the Dutch marine clay soils magnesia ranks first. I found an average of 0.08 gm. exchangeable MgO and 1.34 gm. acid-soluble MgO per 100 gm. soil. In 1920, I suggested two possibilities for the transition of MgO from the acid-soluble form into the exchangeable form, viz., the grinding of the soil particles, resulting in an increase of surface, and a slow diffusion from

the interior of the soil particles towards the surface. In the case of the old Dollard soils I am inclined to think rather of this latter alternative.

With further weathering in still older soils (see soil 8, 380 years old), the content of exchangeable CaO falls still farther, and a further slight increase in the MgO content occurs; but the loss of exchangeable lime is greater, so that the S, V, and pH values now decrease ($S = 30.0$; $V = 32.1$; pH = 5.9). The relative proportion now becomes $71 + 24 + 2 + 3 = 100$.

Soils older than no. 8 (380 years) have not yet been so thoroughly investigated. As far as is known, however, pH values below 5 seldom, if ever, occur in older soils of this type.

THE MINERAL ADSORBING SOIL COMPLEX

Finally, I would point out that during this entire weathering process of the Dollard clay soils over a period of nearly 400 years in the temperate, humid Dutch climate, virtually no change takes place in the mineral adsorbing soil complex. In the oldest marine clay soils this complex still has practically the same composition as in the youngest polder soils. I consider myself justified in ascribing this to the lack of appreciable quantities of acid humus substances, that is, the substances, which, in the cold and temperate humid climates, attack the mineral adsorbing soil complex. As a result of the leaching of the soil water containing acid humus, the constituents Al_2O_3, SiO_2, and Fe_2O_3 of the mineral adsorbing complex are carried off and only the practically unweatherable minerals—quartz and mica—remain (15).

CONCLUDING REMARKS

The foregoing discussion in no way exhausts the subject. Important investigations have been carried out on such phases as the transformation of the humus substance, on the availability of the nitrogen compounds and the P_2O_5 compounds in the youngest soils, and the decrease of the P_2O_5 content and the K_2O content in the oldest soils. Even a brief mention of these questions is impossible within the scope of this article. I will conclude with a remark of a practical nature. Liming tests on the oldest Dollard soils (table 4, soil 8) have shown that of the excess lime applied only enough is adsorbed in exchangeable form to raise the S, V, and pH values to about 40, 43, and 7.4, respectively. With presscake[4] this takes place very soon after fertilizing, that is, in about one year; burnt lime took about 2 years. Higher values than these are not reached, in spite of the fact that there is still an excess of lime, which excess changes in the soil into $CaCO_3$ (8, 17). During the adsorption of the calcium, only hydrogen, and not exchangeable magnesium, is replaced by the calcium. Should, therefore, the occurrence of exchangeable MgO in quantities such as those observed in soils 6, 7, and 8 (table 6) be detrimental either to the soil structure or to the plant growth, the soil must be

[4] A by-product of sugar refining, called, in German, *Kreideschlamm*.

limed before the increase of exchangeable MgO begins; that is, while the soil still contains small quantities of $CaCO_3$.

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INFLUENCE OF MANURE, IRRIGATION, AND CROPPING PRACTICES UPON SOIL MICROBIOLOGICAL ACTIVITIES[1]

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The beneficial influence of manuring, irrigation, and cropping practices upon the productivity of a highly calcareous soil as measured in terms of crop yield have been determined in long-time experiments conducted at the Utah Agricultural Experiment Station (5, 6, 7, 10). It was also shown in the early stages of the field experiments that barnyard manure and irrigation water modified the microflora of the soil so that a close relationship existed between the bacterial numbers and the ammonifying, the nitrifying, and the crop-producing powers of the soil (4). It is the purpose of this work to make a more extensive study of the microbiological activities of this soil after it had been under known field treatments for a period of 21 years.

The work was done on soil from two series of plats (5) at the Greenville Experimental Farm, one series having been fallowed and the other cropped but otherwise given the same treatments, which were as follows, on the acre basis:

IRRIGATION WATER	MANURE
inches	*tons*
0	0
20	0
40	0
0	5
20	5
40	5
0	15
20	15
40	15

SAMPLING

The surface soil was removed to a depth of one-half inch, and samples were taken by auger to a depth of 8 inches. Twenty-five borings from each plat

[1] Contribution from the department of chemistry and bacteriology, Utah Agricultural Experiment Station. Publication authorized by director.

[2] Associate bacteriologist. The author is indebted to J. E. Greaves for valuable assistance in the planning and preparation of this study.

were made at random points to assure more uniform sampling of the plats (8). The cropped plats were sampled midway between the rows of corn, to avoid too strong an influence from the plant roots (2). The samples were placed in sterilized ore sacks, mixed well, and sieved. Microbiological count studies were begun immediately. All results except counts of microörganisms are recorded on the oven-dry basis.

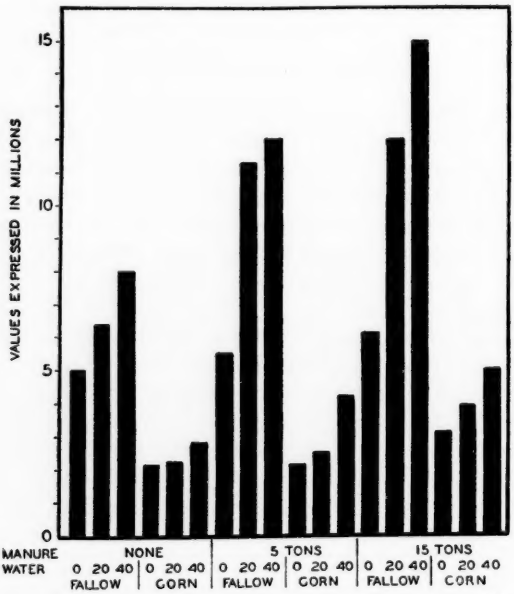
VALUES EXPRESSED IN MILLIONS
15
10
5
0
MANURE
WATER
NONE
5 TONS
15 TONS
0 20 40 | 0 20 40 | 0 20 40 | 0 20 40 | 0 20 40 | 0 20 40
FALLOW | CORN | FALLOW | CORN | FALLOW | CORN

FIG. 1. NUMBERS OF BACTERIA AND ACTINOMYCES IN SOILS AS INFLUENCED BY FALLOW VS. CROPPING, BY MANURE, AND BY IRRIGATION

METHODS AND RESULTS

Numbers of microörganisms

The numbers of bacteria and actinomyces were determined by the plate count on a sodium albuminate agar (3). The mold count was made upon a peptone-glucose acid agar (3). Ten-gram portions of soil from each sample were weighed into 90-cc. water blanks and the contents shaken vigorously for 5 minutes. Subsequent dilutions of 1/10,000, 1/50,000, and 1/100,000 were used for making counts of bacteria and actinomyces. The most uniform values were found at the highest dilution, and the results shown in figure 1 are based upon the average from six plates. An incubation period of 7 days at 25–28°C. was used. The mold count was made from dilutions of 1/100, 1/1,000, and 1/10,000, and the results given in figure 2 are on the basis of the average of

six plates at the intermediate dilution, which gave uniform values. These plates were incubated from 4 to 7 days at 25–28°C.

The numbers of bacteria and actinomyces are higher on the fallowed soils than on the cropped soils. This is in comparative agreement with the results found in the early period of the field experiment (4). The counts increased at all three levels of irrigation as the quantity of manure applied increased; also within each manured group as the amount of water increased. This indicates the favorable influence of increased quantities of water and of manure upon the numbers of bacteria and actinomyces in these soil samples.

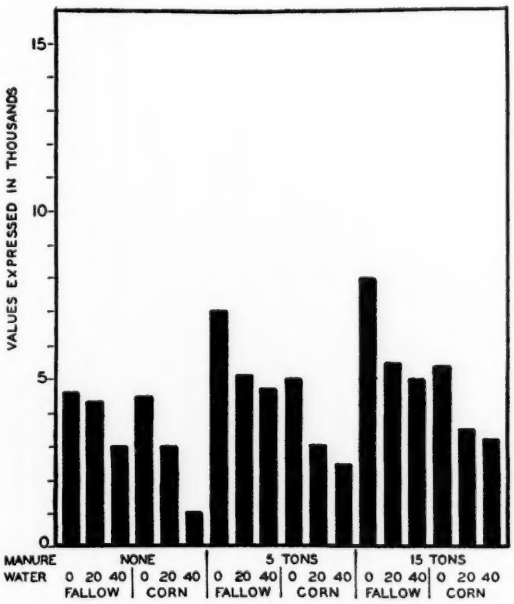

FIG. 2. NUMBERS OF MOLDS IN SOILS AS INFLUENCED BY FALLOW VS. CROPPING, BY MANURE AND BY IRRIGATION

The results from the enumeration of fungi differed from the counts of bacteria and actinomyces in that the numbers of fungi decreased as the amount of water applied increased. This inverse proportion is consistent and shows that the fungi are not favored by the higher quantities of moisture applied. This is in accord with the general knowledge that filamentous fungi have different optimum moisture requirements than those of bacteria. The influence of manure upon the mold count is considerable, though greatly offset by the influence of increased moisture. The fallowed plats gave a higher count than that of the cropped plats.

Evolution of carbon dioxide

In a mixed population of heterotrophic aerobic microörganisms, carbon dioxide is evolved by all. The quantity produced from a soil in a given period of time, however, is dependent upon certain factors, as pointed out by Stoklasa (11). Before any interpretation, therefore, can be placed upon the results of carbon dioxide formation, consideration must be given to these factors. They

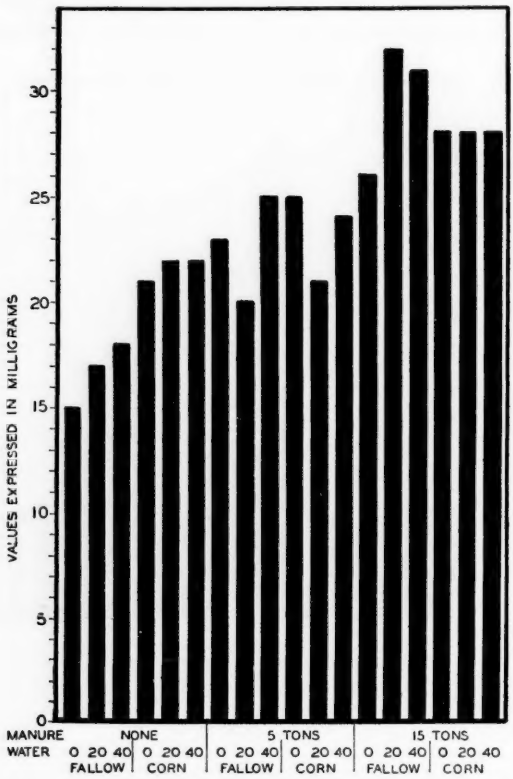

FIG. 3. CARBON DIOXIDE EVOLVED FROM 100 GM. OF SOIL AS INFLUENCED BY FALLOW VS. CROPPING, BY MANURE, AND BY IRRIGATION

are (*a*) number and kind of microörganisms present; (*b*) amount, composition, and degree of decomposition of organic matter present; (*c*) soil aeration; (*d*) moisture content; (*e*) physical nature of the soil; (*f*) chemical composition (modified by fertilizers); (*g*) soil reaction; and (*h*) types of plants grown. The sum total of these factors will influence the rate of carbon dioxide evolution. Under these known conditions this rate will be an index of soil fertility.

A study of CO_2 formation was applied to these soil samples in measuring the evolution of carbon dioxide from the soil alone, from the soil plus dextrose, and from the soil plus cellulose. This was done, however, in the absence of a knowledge of the influence of some of the aforementioned factors. The results obtained are therefore not given so full an interpretation as might otherwise be justified.

In the study of CO_2 formation by the soil alone, 100-gm. portions of the fresh sieved soil were placed in duplicate in long-necked 500-cc. flasks, and the moisture content was adjusted to 50 per cent of the total moisture-holding capacity of the soils. These flasks were set up in a grouping arrangement as outlined by Waksman and Starkey (14) and were kept in a constant temperature room (25–28°C.) for the period of incubation (18 days). The results of this study, the average of two closely agreeing duplicates, are graphically represented in figure 3. The benefits of irrigation and manure are evident in these results The average of the manure treatments with no water is somewhat greater than the average of the irrigation treatments with no manure, and the values in the cropped soils are greater than those in the fallowed soils. The averages for 5 tons of manure and for 20 inches of water approximate the same value, both for cropped and for fallowed soils.

The influence of dextrose upon these soils was studied in the following manner. In duplicates, 500 mgm. of dextrose was added to 100-gm. portions of soil in flasks. The soil was adjusted to an optimum moisture content, the flasks were set up as noted in the preceding experiment and incubated at 25–28°C. for 72 hours. The carbon dioxide formed was measured at short intervals throughout the incubation period. The results are given in figure 4. Averaging all manured plats upon the basis of each respective irrigation treatment indicates that the ability of the soils to decompose dextrose increases somewhat with the quantity of water applied. Likewise, when all irrigation treatments are averaged, a similar increase is noted in the carbon dioxide evolved as the amount of manure added increased. In almost every case, however, the fallowed soils produced larger quantities of carbon dioxide than did the cropped soils. The lowest result is found in the cropped soil receiving no water and no manure, and the highest value is recorded for the fallowed soil receiving 40 inches of water and 15 tons of manure per acre.

To learn the influence of cellulose upon the rate of carbon dioxide evolution, 1 gm. of ground filter paper (cellulose) was added to 100 gm. of each soil and was well mixed. Samples were prepared in duplicate. The soils were adjusted to optimum moisture conditions and incubated at 25–28°C. for 18 days. The carbon dioxide evolved was measured daily. The results are given in figure 5.

Earlier studies (1) have shown the amount of cellulose decomposed to be dependent upon the quantities of available nitrogen and phosphate in the soil. The more available nitrogen is present in the soil, the greater will be the amount of carbon dioxide liberated through cellulose decomposition.

In the group receiving no manure, the soils in the two series—fallowed and

cropped—did not differ greatly from each other. In this group, an application of 20 inches of water caused a decrease of approximately 25 to 30 per cent in the amount of carbon dioxide produced as against no irrigation; even an application of 40 inches gave a significant decrease. Where 5 tons of manure per acre were added, the results from the fallowed soils are lower where irrigation water was applied at the rate of 0 and 40 inches per acre than are those from the corresponding soils which were cropped. In the group of soils receiving 15 tons of manure per acre, a wide difference exists between the results from all

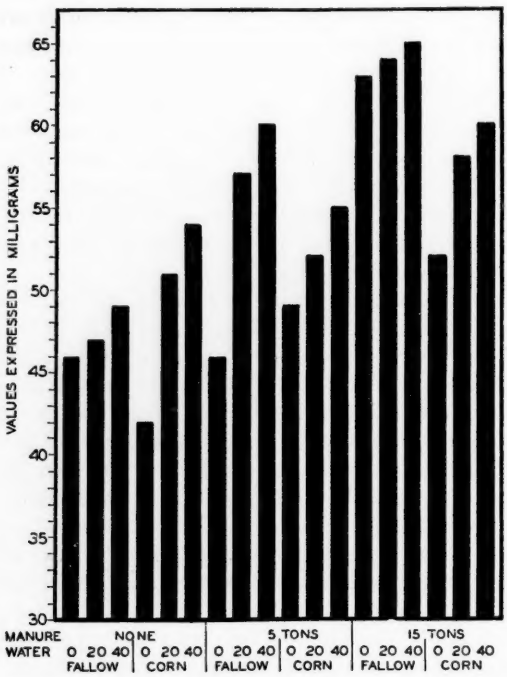

FIG. 4. CARBON DIOXIDE EVOLVED FROM 500 MGM. OF DEXTROSE IN SOILS AS INFLUENCED BY FALLOW VS. CROPPING, BY MANURE, AND BY IRRIGATION

pairings—fallowed and cropped—but is greater where 0 and 40 inches of water were applied than where 20 inches was applied. The graph brings out sharply the stronger influence of manure than of water upon this study.

Nitrate accumulation

The ability of these fallowed and cropped soils to accumulate nitrates was studied by methods suggested by Waksman (12).

In the nitrification of the soil's own nitrogen, a definite quantity of soil

(100 gm.) was kept in the constant temperature room (25–28°C.) for 30 days under optimum moisture conditions. The soil was well mixed at the end of the incubation period, and 50 gm. was shaken for 15 minutes in bottles containing distilled water and lime (CaO). The nitrate content was determined by the phenoldisulfonic acid method (2). The results are given in figure 6.

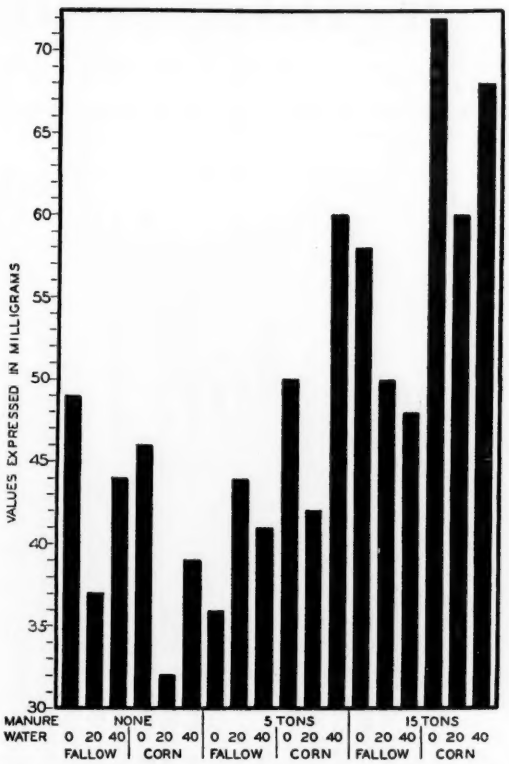

FIG. 5. CARBON DIOXIDE EVOLVED FROM 1 GM. OF CELLULOSE IN SOILS AS INFLUENCED BY FALLOW VS. CROPPING, BY MANURE, AND BY IRRIGATION

This method is used for furnishing information on the forms of nitrogen present in the particular soil and on the speed with which these forms are transformed into nitrates and thus made available for plant growth.

On this basis, the cropped soil receiving 20 inches of water and 15 tons of manure per acre has a maximum nitrate accumulation. In general, the cropped soils showed a greater nitrate accumulation than that of the fallowed soils.

To obtain an index of the nitrifying capacity of soils under optimum reaction

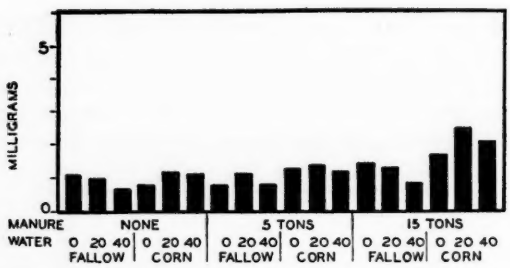

FIG. 6. NITRATE NITROGEN ACCUMULATION IN SOILS AS INFLUENCED BY FALLOW VS. CROPPING, BY MANURE, AND BY IRRIGATION

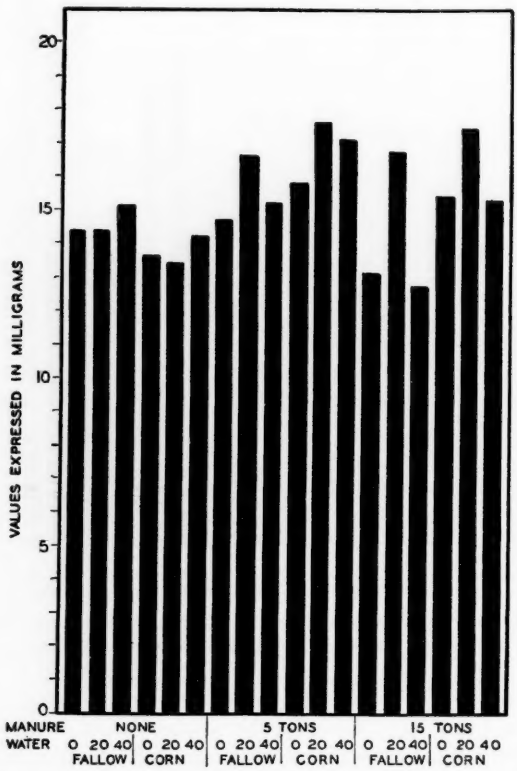

FIG. 7. NITRATE NITROGEN ACCUMULATION FROM AMMONIUM SULFATE IN SOILS AS INFLUENCED BY FALLOW VS. CROPPING, BY MANURE, AND BY IRRIGATION

conditions, a study was made of the nitrification of ammonium sulfate in the soil. Thirty milligrams of nitrogen as ammonium sulfate was added to 100

gm. of each soil in a tumbler and incubated for 30 days at optimum moisture content. Preliminary tests showed it was not necessary to add calcium carbonate to these soils in order to have the nitric and sulfuric acids neutralized. The nitrate determinations were made as usual. Figure 7 illustrates the findings of this study.

Cropped soils receiving 5 tons and 15 tons of manure gave higher values than did the respective fallowed soils, whereas the soils receiving no manure gave values which favored the fallowed soils. Five tons of manure gave the maximum nitrate accumulation for fallowed and cropped soils.

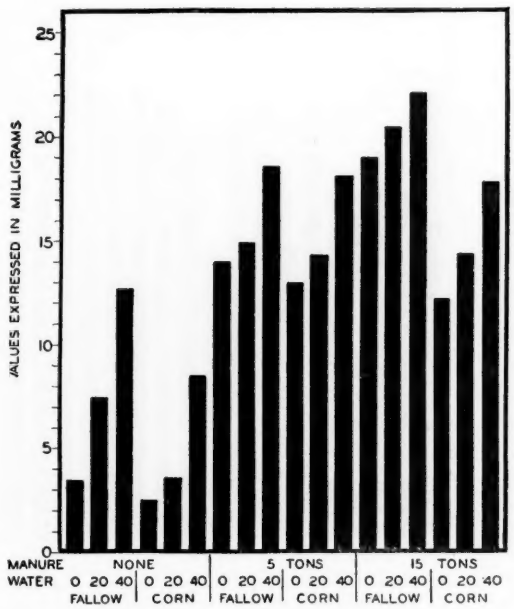

FIG. 8. NITRATE NITROGEN ACCUMULATION FROM DRIED BLOOD IN SOILS AS INFLUENCED BY FALLOW VS. CROPPING, BY MANURE, AND BY IRRIGATION

When the influence of water is considered, 20 inches per acre gave the maximum production of nitrate nitrogen, small differences being noted between 0 and 40 inches.

An organic substance in the form of dried blood in a concentration of 0.25 per cent was well mixed with soil samples, in duplicate, and incubated for 15 days. Figure 8 graphically illustrates these results.

Manure has been especially beneficial to these soils in developing a capacity to transform organic nitrogen compounds into nitrates. The fallowed soils gave higher results than did the cropped soils. The greatest gain is noted in those soils which received manure. Although significant gains appear in favor

of 15 tons over 5 tons of manure, yet the gain of 5 tons of manure over none is the most outstanding.

The value of irrigation has not been highly significant, except in the group of soils receiving no manure.

Nitrogen fixation

The nitrogen-fixing power of the soils was measured by inoculating 5-gm. samples of soil into 100-cc. portions of a standard mannite solution (13) and

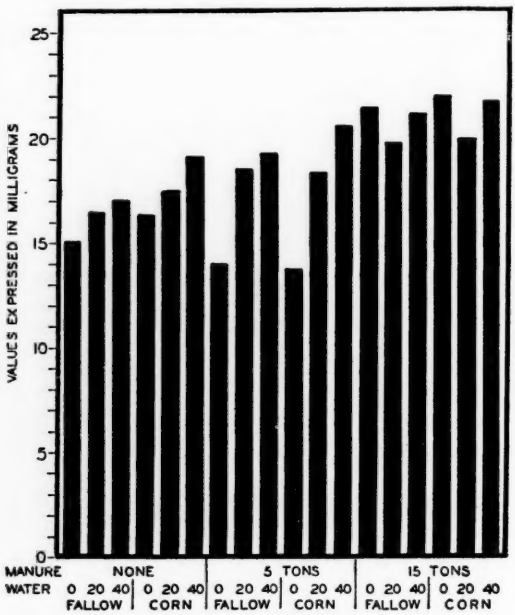

FIG. 9. NITROGEN FIXATION IN SOLUTION AS INFLUENCED BY INOCULATED SOILS VARIABLY CULTIVATED, MANURED, AND IRRIGATED

incubating for 30 days at 25–28°C. The increase in total nitrogen above the control (sterilized solution plus 5 gm. of original soil) signified the nitrogen-fixing ability of the soil or served as an index of the activities of the nitrogen-fixing flora of the soil, as given in figure 9.

The results show some increase due to the addition of larger volumes of irrigation water. The cropped soils exceeded the fallowed soils in the quantity of nitrogen fixed.

The use of manure caused a significant increase in the ability of the soil to add new nitrogen.

CONCLUSION

The results of the microbiological activities in the fallowed and cropped soils are summarized in tables 1 and 2, where the values are given on the

TABLE 1

Comparison of microbiological activities in fallowed soils

ACTIVITIES	MANURE			WATER		
	None	5 tons	15 tons	None	20 inches	40 inches
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Bacteria and actinomyces	100	148	169	100	180	213
Fungi	100	140	155	100	77	65
CO_2 from original soil	100	135	176	100	110	119
CO_2 from dextrose	100	115	136	100	110	111
CO_2 from cellulose	100	93	121	100	92	92
NO_3—N from original soil	100	98	126	100	102	71
NO_3—N from $(NH_4)_2SO_4$	100	106	97	100	113	101
NO_3—nitrogen from dried blood	100	200	260	100	117	146
Nitrogen fixed	100	107	128	100	108	114
Total nitrogen	100	113	130	100	88	95
Organic carbon	100	110	118	100	100	99

TABLE 2

Comparison of microbiological activities in cropped soils

ACTIVITIES	MANURE			WATER		
	None	5 tons	15 tons	None	20 inches	40 inches
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Bacteria and actinomyces	100	121	166	100	121	166
Fungi	100	125	143	100	64	44
CO_2 from original soil	100	104	127	100	92	96
CO_2 from dextrose	100	106	116	100	112	116
CO_2 from cellulose	100	131	172	100	81	100
NO_3—N from original soil	100	121	181	100	134	99
NO_3—N from $(NH_4)_2SO_4$	100	123	117	100	113	104
NO_3—N from dried blood	100	308	401	100	113	140
Nitrogen fixed	100	100	120	100	107	118
Total nitrogen	100	97	126	100	104	89
Organic carbon	100	106	119	100	93	95
Corn *bushels*	100	127	140	100	124	120
Stover *tons*	100	130	157	100	117	119

percentage basis. The values in the unmanured and unirrigated soils are taken as 100 per cent, and the results from the soils receiving manure and irrigation water are given their corresponding percentage values.

The results of the crop in terms of bushels of ear corn and tons of stover are given in table 2 upon the percentage basis for comparative reasons.

These tables are very interesting in the summaries presented of the various microbiological activities and the relationships found existing among them. In almost every case the larger additions of manure reflect an increase among the soil microorganisms. This relationship holds true among both the fallowed and cropped soils. The one exception to be noted is found in the fallowed and the cropped soils in the accumulation of nitrogen from ammonium sulfate, where the soils receiving 5 tons of manure per acre exceeded those receiving 15 tons of manure.

The addition of water to these soils did not prove so generally beneficial to the microbiological activities as did the application of manure. The increased irrigation even lessened some of these activities, as for instance the

TABLE 3

Correlation between crop yield and microbiological activities

MICROBIOLOGICAL ACTIVITY	EAR CORN (BUSHELS)	CORN STOVER (TONS)
	r	r
Numbers of bacteria and actinomyces	0.91 ± 0.04	0.79 ± 0.08
Numbers of fungi	0.67 ± 0.12	0.83 ± 0.07
CO_2 from original soil	0.92 ± 0.03	0.65 ± 0.13
CO_2 from dextrose	0.82 ± 0.07	0.64 ± 0.13
CO_2 from cellulose	0.88 ± 0.05	0.51 ± 0.16
NO_3—N from original soil	0.86 ± 0.06	0.65 ± 0.13
NO_3—N from $(NH_4)_2SO_4$	0.84 ± 0.07	0.59 ± 0.14
NO_3—N from dried blood	0.90 ± 0.04	0.62 ± 0.13
Nitrogen fixed	0.74 ± 0.10	0.92 ± 0.03
Total nitrogen	0.86 ± 0.06	0.73 ± 0.10
Total organic carbon	0.81 ± 0.08	0.76 ± 0.09

numbers of filamentous fungi (molds) and the evolution of CO_2 from cellulose among both the fallowed and cropped soils. There was a decrease in the production of CO_2 and in the total organic carbon content of the original soils at both levels of irrigation given to the cropped soils, and the 40-inch application of water decreased the total nitrogen content over the 20-inch irrigation. The fallowed soils decreased in their total nitrogen content at both levels of irrigation, and no apparent change occurred in the total organic carbon content.

The averages for the fallowed soils of the eleven activities summarized for the two levels of manuring and irrigation have a close ratio to the corresponding averages obtained for the manured soils. The averages from the latter in turn show a similarly close relationship to the crop produced.

RELATIONSHIP BETWEEN CROP YIELD AND MICROBIOLOGICAL ACTIVITIES

The total results of the activities of microorganisms which have been reported herein, were compared with the crop produced in 1931 (the twenty-

first year of continuous cropping), and correlations were obtained which are reported in table 3.

In publishing these comparisons, it is recognized that a considerable chance for error enters this problem where so few samples were used. Only nine experimental plats producing a crop were sampled; nevertheless, it is interesting to note the relationships found.

The correlations are higher for ear corn than for stover. Very significant values are obtained in comparison with ear corn for numbers of bacteria and actinomyces, respiratory and decomposing powers of the soil, nitrate-nitrogen accumulation, and total nitrogen and organic carbon contents. The values for numbers of fungi and for nitrogen fixation are lower but still significant. Fungi decreased as the amount of irrigation increased, and the benefits of manure were not great enough to offset sufficiently the values which gave the low correlation. Nitrogen fixation was not greatly influenced by irrigation or manure and consequently did not correlate so well with the crop yield in terms of ear corn.

The correlation values for corn stover are high enough to be significant. The same relationship does not exist in the values for any microbiological activity between ear corn and corn stover. No attempt, however, is being made to explain this variation.

SUMMARY

Soil samples were taken from experimental field plats which were divided into two series upon the basis of continuous fallow and yearly cropping to corn and which had been treated with varying quantities of manure and irrigation water over a period of 21 years. Manure had been applied at the rate of 0, 5, and 15 tons an acre yearly; and irrigation water had been given in 0-, 20-, and 40-inch quantities. Nine samples were taken from each series.

Studies were made to determine the influence of manure and irrigation water upon certain microbiological activities in these soils; the relationship between these microbiological activities in fallowed and in cropped soils; and the correlation between the crop produced and these microbiological activities.

On the average of all irrigation treatments, the larger quantities of manure in both fallowed and cropped soils increased the total nitrogen and organic carbon contents, numbers of microorganisms, and the power of the soils to decompose organic matter, to accumulate nitrates, and to fix nitrogen.

On averaging all manurial treatments, a tendency was noted for certain microbiological activities to increase as the amount of water applied increased. In the cropped soils these activities include numbers of bacteria and actinomyces, the evolution of CO_2 from dextrose, the accumulation of nitrates, and the fixation of nitrogen. The influence of added water was negative on numbers of filamentous fungi and on evolution of CO_2 from original soil and from added cellulose and was not appreciable on total nitrogen and total organic

carbon content. The microbiological activities in the fallowed soils influenced favorably by irrigation comprise numbers of bacteria and actinomyces, the evolution of CO_2 from original soils and from added dextrose, the accumulation of nitrates, and nitrogen fixation. The addition of water to these soils decreased the numbers of filamentous fungi, the production of CO_2 from cellulose, and the total nitrogen content. The total organic carbon content remained constant.

The numbers of bacteria, actinomyces, and fungi and the quantity of nitrogen fixed in the fallowed soils were higher than those in the cropped soils under both irrigation and manuring. The accumulation of nitrates was favored in the cropped soils.

Under the influence of manure the fallowed soils exceeded the cropped soils in the production of CO_2 from the original soil and from added dextrose. No apparent differences existed in the total organic carbon content of these soils.

The influence of irrigation was greatest in the fallowed soils on the numbers of microorganisms, the evolution of CO_2 from cellulose, nitrogen fixation, and total organic carbon. Irrigation favored the cropped soils in CO_2 evolution from original soils and from added dextrose but had little if any influence on the accumulation of nitrates from ammonium sulfate and dried blood, the fixation of nitrogen, and the total nitrogen content.

The results of the crop for 1931 show a good correlation with the microbiological studies made on the soils.

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COMPOSITION OF SOYBEAN NODULES AND ROOT NODULE BACTERIA[1]

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Few studies have been conducted on the chemical composition of either of the symbionts concerned in biological nitrogen fixation through association of the root nodule bacteria (*Rhizobium* sp.) and leguminous plants. Such information is becoming necessary both for its intrinsic value and for its integration with the results of other types of studies on the symbiotic nitrogen fixation problem (physiological, physicochemical), which are already far in advance.

Knowledge of the chemical composition of the bacteria with respect to the nitrogenous constituents is desirable not only for comparison with the chemical composition of nodules, but in addition, to determine whether differences in physiological characteristics can be correlated in any way with differences in chemical components. Probably of greater interest is information which deals with the nature of the nitrogen fractions present in the root nodule, since this tissue is apparently the seat of the symbiotic nitrogen fixation process. Although it appears improbable that the analytical methods now available will suffice to distinguish the nitrogen compounds resulting from the fixation process from those concerned in the many other phases of the life of both bacteria and plant, knowledge of the composition of the cells involved should be of value in interpretation of data from other types of studies concerned with the mechanism of the fixation process.

Lack of information arises in part from the difficulty of obtaining sufficient material for analysis by available methods. The preparation of artificial inoculum for leguminous plants on a commercial scale at the University of Wisconsin, together with the development of semimicrotechnic for the analysis of nitrogenous components of biological tissues (18), has provided materials and methods for studies on the chemical composition of several species of the root nodule bacteria as well as detailed analyses of nodules of the soybean.

Three general approaches to the problem comprise microchemical studies on the structures of the cells through use of cytological technic; isolation and characterization of groups of tissue constituents, *e. g.*, nitrogen extracted with

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NaCl solutions; and analysis of the tissue as such without the isolation of general groups. In this paper, results obtained by the third method are discussed. For convenience the studies have been divided into four sections, as follows: comparison of the nitrogen components of four species of the bacteria; comparison of the soluble and condensed forms of nitrogen in the tops, roots, and nodules of soybeans actively fixing nitrogen; detailed analysis of the nodular tissue of soybeans, the entire dried tissue being used; similar analysis of the soluble portion of fresh nodules.

NITROGEN DISTRIBUTION OF THE ROOT NODULE BACTERIA

Four species of the root nodule bacteria belonging to different cross-inoculation groups—*Rh. trifolii* (clover), *Rh. leguminosarum* (pea), *Rh. meliloti* (alfalfa), and *Rh. japonicum* (soybean)—have been analyzed for their nitrogen constituents.

The bacteria were grown in 200-cc. and 400-cc. flat bottles on a medium composed of sucrose, 15 gm.; K_2HPO_4, 0.5 gm.; $MgSO_4$, 0.2 gm.; NaCl, 0.1 gm.; $CaCO_3$, 3.0 gm.; yeast water (1:10), 100 cc.; agar, 15 gm.; and water, 900 cc. Details of the preparation, inoculation, and incubation have been described elsewhere (11). The medium used for the soybean differed from the aforementioned medium in that sucrose was replaced by mannitol. Each bottle was inoculated with a mixture of three of the most efficient strains of the particular species, all obtained from the Wisconsin collection. The cultures were incubated for approximately 17 weeks at room temperature (25–30°C.).

The bacteria were washed from the cultures with sterile water, filtered through cheesecloth to remove any agar lumps, and the cells centrifuged from the suspension. These were resuspended in warm, sterile water and re-centrifuged, the process being repeated four times, after which the nitrogen content of the bacteria remained constant, though considerable amounts of gum remained attached to the cells. Microscopical examination revealed no contamination.

The bacteria were then evenly suspended in sterile water to a definite volume, and aliquots were taken for dry weight, total nitrogen, acid hydrolysis, alkaline hydrolysis, and tryptophane determinations. *Dry weight* was determined by drying to constant weight at 102°C. *Total nitrogen* was determined by the semimicromethod of Umbreit and Bond (23). On the portion of the suspension taken for acid hydrolysis (20 per cent HCl, 24 hours, biuret negative), the humin formed was filtered off, and nitrogen was determined on it. The filtrate and washings after the removal of the humin were concentrated under reduced pressure so that 1 cc. of the hydrolyzate contained approximately 1 mgm. of nitrogen. Nitrogen distributions were determined on 20 cc. of the acid hydrolyzate by the method of Orcutt and Wilson (18) which was slightly modified to permit acid hydrolysis. Essentially the method determines *humin* nitrogen, *amide and* NH_3 nitrogen, *basic* nitrogen (25), and *non-basic* nitrogen, all by direct methods.

Cystine was determined on a Neuberg precipitate (15) of the acid hydrolyzate by a modification of the Folin method described by Cavett (4), since results obtained by the direct alkaline hydrolysis method of Zahnd and Clarke (30) were erratic. *Tryptophane* was determined on the original suspension by the method of Holm and Greenbank (12). Histidine could not be detected by the Kapeller-Adler reaction (13), but the diazo reaction (4) would tend to indicate that some was present, though accurate determinations could not be obtained. These reactions served to show that histidine was probably rather low in all organisms examined. Basic amino and nonbasic amino nitrogen were determined as described by Orcutt and Wilson (18), but the results were not consistent. They tended to indicate, however, that the pea and clover

TABLE 1

Nitrogen distributions on four species of the root nodule bacteria

	RH. MELILOTH (ALFALFA)			RH. JAPONICUM (SOYBEAN)	RH. TRIPOLII CLOVER	RH. LEGUMINOSARUM (PEA)
Age of culture.....*weeks*	17.8			15.6	14.8	17.6
Total nitrogen...*per cent dry weight*	8.4			9.9	2.0*	6.4*
Nitrogen distribution (expressed as per cent of total N)	*a*	*b*	*c*			
Humin-N.....	2.94	3.15	2.65	1.62	4.57	0.81
Amide + NH_3.....	10.6	10.8	10.6	12.5	12.2	12.6
Basic-N.....	29.4	23.2	25.6	24.8	22.4	24.5
Nonbasic-N.....	59.5	57.4	58.4	60.6	67.8	63.5
Total N.....	102.5	94.5	96.2	98.9	107.0	101.4
Cystine-N.....	1.51	.....	.....	1.62	0.93	0.96
Tryptophane-N.....*per cent total N*	2.77	.....	.....	1.51	0.41	0.80

* Heavy gum producers.

organisms had a lower amino/nonamino ratio than the other two species. In the soybean organism the amino/nonamino ratio was about 1.

The data are summarized in table 1; the nitrogen distributions were run in triplicate on each hydrolyzate, the figures in the table being the average of the three. Normally, the agreement was well within 5 per cent. In addition, data on three separate hydrolyses are given for the alfalfa organisms to indicate the reproducibility of the results. The analyses presented represent a net composite of the various strains and ages of each species and as such can be interpreted only in general terms. The chemical composition of bacteria will vary with media and with age of cultures (2) but these factors, if maintained constant for all species, would not be important in comparative studies. The medium used in this work has been shown (10) to cause little if any change in the biological characters of the organisms (physiology, ability to infect plant

and to fix nitrogen) even after long cultivation. Also, the bacteria analyzed had reached their maximum growth and were in their stationary phases; in this state they still retain the ability to invade the plant, form nodules, and fix nitrogen, and they show little loss in viability. For these reasons it is apparent that, should there be any differences among the species large enough to be detectable by chemical means, these differences should be apparent in the composite culture analyzed. Because of the general crudeness of the present methods of nitrogen analysis when applied to biological tissue, any factors in composition correlated with physiological processes would have to be fairly large (of the order of 10 per cent) to be detected with certainty.

Because of the varying amounts of gum produced, particularly on media containing carbohydrate, the *percentage nitrogen* is of little value. Of the strains examined the clover and pea organisms (heavy gum producers) contained a much lower percentage of nitrogen than did the alfalfa (moderate gum) or soybean (very little gum). Since the gum is but slightly soluble in water, it was not entirely removed when the cells were washed, and variations in gum production are reflected in the percentage nitrogen. The gum itself is nitrogen free and would therefore not enter into the nitrogen distributions.

From a consideration of these facts it appears that there are no significant differences in the main fractions determined; *amide and* NH_3, *basic*, and *non-basic* nitrogen being essentially the same for all species examined.

By a comparison of the humin nitrogen obtained in the acid hydrolysis with the amount of tryptophane present, it seems probable that most of the humin originated solely from the tryptophane, and, with the exception of the clover organism, which was high in carbohydrate gum as evidenced by its percentage nitrogen, little further decomposition occurred. The values found for the amide fraction in these organisms are comparable to those reported for other bacteria; it is noted, however, that the absolute value in any case is probably high because of splitting of NH_3 from amino acids during the drastic acid hydrolysis.

On the basis of the data revealed by present available methods for determining nitrogen constituents in proteins, there is little evidence that species differences in the root nodule bacteria can be correlated with chemical composition. Although the pea and clover organisms, which resemble each other in some physiological characteristics, both have a lower content of cystine and tryptophane than do the other two species, and, as has been mentioned, appear to differ somewhat in the amino/nonamino ratio of the basic fraction, the outstanding feature of the analyses is the constancy of the general composition of the root nodule bacteria irrespective of the cross-inoculation grouping.

COMPARISON OF NITROGEN FRACTIONS IN DIFFERENT PORTIONS OF THE SOYBEAN PLANT

The data of Strowd (20) on the forms of nitrogen in soybean nodules indicated that this tissue was high in basic nitrogen. It has been suggested that this fraction might be concerned in the nitrogen fixation process, par-

ticularly since it seems from Strowd's analyses of roots, stems, and tops to be moving from the nodule into the plant. Metabolic studies made by Orcutt (16) on the nitrogenous components in the soluble portion from nodules, roots, stems, and leaves failed to establish such movement and, in general, failed to confirm the high basic nitrogen in the nodules. The two sets of data, however, are not strictly comparable, since Orcutt's studies dealt with the soluble portion only, whereas those of Strowd were concerned with the composition of the entire nodule. Because of the inconsistent nature of the available data it appeared desirable to obtain further results on this question of comparative composition of nodules and other organs of the plant.

Nitrogen distributions were made on both the entire fresh tissue after hydrolysis for 24 hours with 20 per cent HCl and on the sap after emzymatic

TABLE 2

Nitrogen components in the root nodules of soybeans compared with other portions of the plant

Fresh tissue; 61 days from planting

FORM OF NITROGEN	ENTIRE TISSUE*			SOLUBLE PORTION†		
	Tops	Roots	Nodules	Tops	Roots	Nodules
Humin-N	7.7	8.7	4.3	....	.....	....
Amide + NH_3	7.3	6.7	11.5	11.3	16.6	14.1
Basic-N	27.2	27.6	28.8	32.9	27.8	35.3
Amino	13.6	13.5	15.6	16.3	15.0	14.6
Nonamino	13.6	14.1	13.2	16.6	12.8	20.7
Nonbasic-N	65.0	66.6	54.7	46.8	59.3	40.9
Amino	46.7	50.0	47.3	34.7	45.3	30.3
Nonamino	18.3	16.6	7.4	12.1	14.0	10.6
Total	107.2	109.6	99.3	91.0	103.7	90.3

* After hydrolysis for 24 hours with 20 per cent HCl.

† After enzymatic hydrolysis.

hydrolysis (18). The analyses were made in triplicate on each portion; the agreement was usually within 5 per cent of the total involved in the determination. The results summarized in table 2 are the averages of the three determinations.

Considering first the analysis of the entire tissue, it is noted that as far as the basic fraction is concerned the content of the nodule does not differ from that in the rest of the plant. Essentially the same may be said for the soluble portion except that, as previously noted by Orcutt (16), the basic nitrogen in the nodule sap bears a ratio of nonamino to amino nitrogen different from that in the remainder of the tissue.

In general, it is concluded that the major nitrogen fractions of both the entire tissue and its soluble forms are virtually alike throughout the plant. Apparently the nodule contains no unique components, with the possible

exception of nonamino basic nitrogen. The need for more information with respect to this fraction prompted detailed analysis of the nitrogen components in the nodule.

DETAILED FRACTIONATION OF NITROGEN COMPONENTS IN DRIED NODULES

Nodules from plants of experiment I (harvest 4) previously discussed by Umbreit and Fred (24), were dried at 80°C. for 56 hours and preserved in a stoppered bottle. The scheme of analysis employed, together with results from the major fractions, are given in table 3. In certain of the separations some of the nitrogen was lost, which is apparent when the nitrogen recovered after a given operation does not equal the total taken. Such losses were usually about 10 per cent of the total involved in the operation. Standard methods (1, 14) of separation were used, and the details will not be given here; however, notes on the methods and further observations are recorded in the following summary, in which the letters in parentheses refer to corresponding letters in table 3:

(*a*) *Hydrolysis.* During hydrolysis considerable amounts of furfural were evolved. Under these conditions the humin formed probably originates from sources other than solely tryptophane and tyrosine (8).

(*b*) *Micronitrogen distributions.* The method of Orcutt and Wilson (18) was slightly modified to permit acid hydrolysis. The results were:

Fraction	*Per cent of N in hydrolyzate*	*Per cent of total N*
Humin	.....	8.63
Amide and NH_3	9.35	8.52
Basic	28.63	26.25
Amino	13.63	12.40
Nonamino	15.00	13.85
Nonbasic	60.60	55.40
Amino	59.62	54.5
Nonamino	0.98	0.9
	98.58	98.80

In this distribution the nonbasic, nonamino ("other") nitrogen figures are abnormally low (because of errors in α amino N determinations), since normally 7 to 10 per cent of the nitrogen is found in this fraction. Actually 7 per cent of proline (which is a constituent of this fraction) was subsequently found among the monoamino acids (see note *f*).

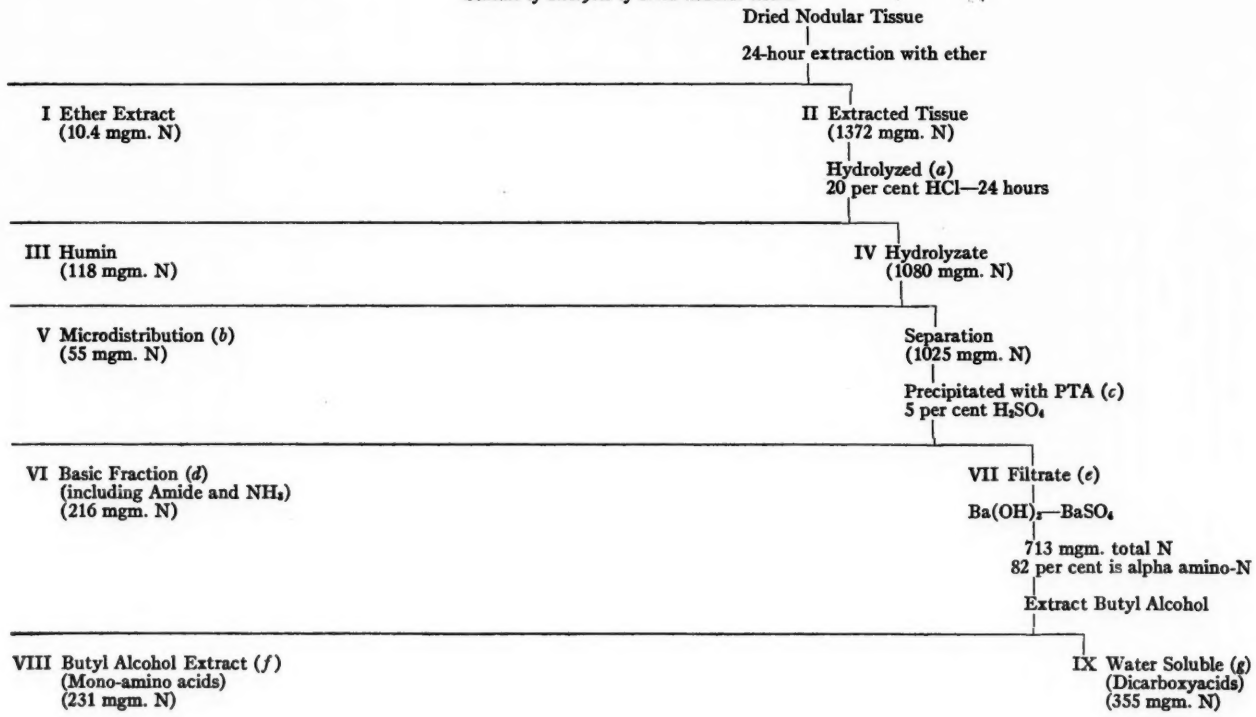
(*c*) *PTA precipitation* (amide and NH_3 were not removed before precipitation). The precipitate contained 216 mgm. N (21 per cent of the total N) in the hydrolyzate. Subsequently 61 mgm. of basic nitrogen together with 29.0 mgm. of NH_3-N were discovered in the "dicarboxylic acid" fraction (IX). The incomplete precipitation of basic nitrogen with PTA on hydrolyzates of whole plant tissue has been noted by other workers (7). In the further examination of the basic fraction this 90 mgm. of PTA precipitable N subsequently found in the "dicarboxylic acid" fraction was not included.

(*d*) *Basic fraction.* PTA was removed with amyl alcohol-ether mixture (27). Difficulty was experienced in obtaining sharp separation because of insoluble matter collecting at the interface.

Analysis of the basic fraction was conducted with the method outlined by Cavett (4) in

TABLE 3

Scheme of analysis of dried nodular tissue



his modification of the Van Slyke (26) procedure. Briefly this is: amide N by aeration, arginine[2] by strong alkali digestion and distillation, cystine with the uric acid reagent of Folin and Marenzi, histidine by the diazo reaction, and lysine by difference. The results are given under *basic nitrogen* in table 4.

The Kapeller-Adler test for histidine (13) gave negative results. When histidine was added to the basic solution a color still failed to develop, indicating an interfering substance. The figure given for histidine is that obtained from the diazo reaction admittedly less specific. It serves to show, however, that histidine is relatively low in the tissue. The test for pyrimidines with Br_2 and $Ba(OH)_2$ was negative.

Attempts were made to separate the basic nitrogen further by fractional precipitation of the silver salts of the basic amino acids (28), but the small quantities of nitrogen available rendered errors due to solubility too great to warrant the attachment of much significance to the data.

(*e*) *PTA filtrate.* PTA was removed by $Ba(OH)_2$, then excess barium was removed by H_2SO_4. Three aliquots containing 20 mgm. N each were extracted with butyl alcohol (5) in Kutscher and Steudel extractors for 36 hours at pH values of 4.0, 6.7, and 8.9; the greatest extraction of nitrogen was obtained at pH 6.7. The main fraction was then adjusted to pH 7 and extracted for 60 hours at atmospheric pressure. The butyl alcohol extract contained 231 mgm. N, and the remaining water solution ("dicarboxylic acid" fraction) contained 355 mgm. N.

(*f*) *Butyl alcohol extract (mono-amino acids).* By vacuum distillation, the butyl alcohol was removed from the extract and the residue was dissolved in water. An insoluble "scum," (35.8 mgm. N) probably water-insoluble diketopiperazines, was formed during the extraction. The water solution after the removal of the "scum" contained 196 mgm. N, of which 127 was alpha-amino nitrogen; the nonamino nitrogen, 69 mgm., constituting 35 per cent of the fraction, was assumed to be proline. This was confirmed by evaporating the solution to dryness *in vacuo* and extracting with absolute alcohol (14) in which proline is soluble. The alcohol extract contained the entire 69 mgm. nonamino nitrogen (proline) and in addition 64 mgm. of alpha-amino nitrogen. From these data it appears probable that the nonbasic nonamino ("other") nitrogen, which appears in increasing quantities as the plant ages (16), is due to proline.

After this separation the ethyl alcohol soluble and insoluble fractions were combined and subjected to a copper salt separation (3, 22). In this procedure the copper salts of the amino acids are separated by their differing solubilities in water and methyl alcohol. The results of this separation were:

	Per cent of fraction
Water insoluble—leucine, phenylalanine, norleucine (?), and methionine	3.9
Water soluble—	
MeOH insoluble—glycine, alanine, serine, tyrosine	10.0
MeOH soluble—valine, isoleucine, proline, hydroxyproline	65.5
Unaccounted for	20.6

(*g*) *"Dicarboxylic Acid" Fraction.* This fraction contained a total of 355 mgm. N of which 320 mgm. was alpha-amino nitrogen. The barium salts of dicarboxylic amino acids (9) precipitated 88 mgm. N in alcohol solution; 29 mgm. was removed by aeration (NH_3); 61

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[2] Throughout this paper the nitrogen recoverable by strong alkaline digestion will be referred to as "arginine" nitrogen. It is recognized, of course, that the procedure used would include any other relatively easily hydrolyzed base, *e.g.*, purine compounds. The conclusions reached regarding "arginine" necessarily can not be definitely limited to this particular amino acid but would include any organic nitrogen base (precipitated by PTA) which liberates all or part of its nitrogen on digestion with strong alkali.

mgm. was further precipitated by PTA (see note *c*); 112 mgm. N was not affected by these treatments, leaving 65 mgm. lost in the operations. Though the error in these separations is high, it is of interest that the dicarboxylic amino acids precipitated by barium comprise 8.4 per cent of the total nitrogen in the hydrolyzate. As the semimicrodistribution indicated that the amide plus NH_3 fraction comprised 9.35 per cent of the total nitrogen (note *b*), it is reasonable to suppose that the amino acids precipitated by barium were aspartic and glutamic which originated from asparagine and glutamine.

The data are summarized in table 4. This table is based upon the assumption that the fractions actually separated were representative of the tissue.

TABLE 4

Summary of analyses on dried soybean nodules

FRACTION	AS PER CENT OF FRACTION	PER CENT OF TOTAL N
Humin-N	....	8.63*
Basic-N. (incl. amide + NH_3)	....	34.8*
Ammonia†	21.1	7.3
Arginine	59.5	20.7
Histidine	7.4	2.6
Cystine	4.7	1.6
Lysine	7.3	2.5
Nonbasic-N	....	55.4*
1. BuOH extract (mono-amino acids)	39.5	21.8
"Diketopiperazines"	10.0	2.2
Cu salts—water-insoluble	3.5	0.8
Cu salts—MeOH-insoluble	9.0	1.9
Cu salts—MeOH-soluble		
Proline	35.0	7.7
Others	24.0	5.2
Unaccounted for	18.5	4.0
2. Non-extracted	60.5	33.6
NH_3	7.8	2.8
Basic	17.2	5.8
Di-carboxylic	24.8	8.4
Other	31.6	10.5
Unaccounted for	18.6	6.1

* From microdistribution data (note *b* to table 3).

† From amide nitrogen originally present as amide (see note *d* to table 3).

For example, the microdistribution indicated that 34.8 per cent of the total nitrogen in the tissue was precipitable by PTA (NH_3 + basic N); actually only 21.1 per cent was recovered because of losses in the separation.

If the 21.1 per cent separated be representative of the 34.8 per cent actually present any component of it (*e.g.*, arginine $\doteq$ 59.5 per cent of separated basic fraction) should comprise the same proportion of the original tissue, *i.e.*, arginine = 59.5 per cent of 34.8 per cent or 20.7 per cent of entire tissue. Although the validity of the assumption may be somewhat doubtful, the errors

introduced are probably not over 10 per cent, and for the purpose of the conclusions drawn this type of presentation gives an adequate summary.

From the data discussed in this section, the following conclusions may be drawn:

The dried nodular tissue contains a wide variety of amino acids, being reasonably similar to other plant tissues.

The basic amino acids (precipitable with PTA) show a high proportion of arginine; *i.e.*, nitrogen liberated by strong alkali digestion, whereas other basic acids are relatively low.

The nonbasic amino acids are characterized by a fairly high content of proline. It seems likely that the nonamino nonbasic nitrogen fraction, which other workers (16, 29) have noted in increasing quantities as the plant ages, is composed primarily of proline.

DETAILED FRACTIONATION OF THE NITROGEN COMPONENTS IN THE SOLUBLE PORTION OF FRESH NODULAR TISSUE

Because of the unusual ratio of nonamino to amino basic nitrogen found in the soluble portion of nodular tissue, attempts were made to separate the components in this fraction. Also, confirmation of the findings reported in the preceding section appeared advisable, fresh tissue being used in order to eliminate changes which accompany drying (19, 21).

Since nodules represent only a small portion of the plant, the quantity of nodular material obtainable in the fresh state becomes an appreciable factor in the separation. The soybeans were grown on nitrogen-poor sand (in 2-gallon glazed jars), watered with tap water, and supplied with Crone's N-free nutrient, the same technic described by Orcutt and Fred (17) being used. The cultures were planted on May 22, 1936, and inoculated with a mixture of three efficient strains of *Rh. japonicum*. At harvest on July 22, 1936, 1,390 gm. fresh nodules (dry weight = 16 per cent) were first ground, suspended in water, homogenized, heated to 70°C (to stop enzyme action), and then pressed through fine mesh cheesecloth. This procedure removed most of the purely structural components and large aggregates. The milky exudate contained insoluble bodies which were suspended as gummy colloids and were very difficult to remove except by long centrifuging and successive filtrations of the supernatant liquid through packed paper pulp. After this clarification the remaining clear brown liquid probably represented the true soluble portion of the tissue.

In an effort to determine the nitrogen components of this soluble portion with respect to the condensed forms, these three physical fractions were hydrolyzed separately (20 per cent HCl, 24 hours, biuret negative), and nitrogen distributions were determined on each. The data indicated that the composition of the three fractions was similar except that the residual material and the colloidally suspended matter were slightly higher in basic nitrogen, and amide nitrogen was higher in the soluble portion.

The scheme of separation applied to the soluble portion (table 5) was modified from that employed in the analysis of the dried tissue to include a Neuberg

precipitation (15). Notes on the methods and further observations are given in the following summary, in which the letters in parenthesis refer to corresponding letters in table 5:

(*a*) *Humin nitrogen.* Again considerable quantities of furfural were evolved, indicating that the origin of the humin was not solely tyrosine and tryptophane.

(*b*) *Microdistribution.* The microdistribution determined by the method of Orcutt and Wilson (18) follows:

Nitrogen Fraction	*Per cent of N in hydrolyzate*	*Per cent of total N*
Humin	....	4.3
Amide and NH_3	11.9	11.5
Basic	30.1	28.8
Amino	16.3	15.6
Nonamino	13.8	13.2
Nonbasic	57.0	54.7
Amino	49.4	47.3
Nonamino	7.6	7.4

It is probable that the amino/nonamino ratio in the basic fraction is incorrect. Considerable difficulty was experienced in obtaining consistent results for the alpha-amino nitrogen in this fraction, presumably because of interfering substances which apparently were concentrated in the Neuberg filtrate in the separation procedure (see note *d*). As is indicated in note *g*, considerable quantities of "arginine" were found in the basic fraction, which should contribute to a lower amino/nonamino ratio than this distribution shows.

(*c*) *Amide and NH_3 Nitrogen.* The experience with the dried tissue indicated the desirability of removing the NH_3 before separation of the other constituents. The hydrolyzate was therefore adjusted to pH 7.5–7.8 and aerated vigorously for 12 hours (until only traces of NH_3 could be detected by Nessler's reagent in the outcoming air).

(*d*) *Neuberg filtrate.* This portion contained a large amount of inorganic salts and accordingly was evaporated to dryness *in vacuo*. The resulting solids were subjected to Soxhlet extraction with absolute alcohol for 24 hours in order to remove nitrogen compounds from the inorganic salts. In the extract, 41 mgm. N, 52 per cent of the fraction, was recovered. Alpha-amino nitrogen determinations were very erratic presumably because of the presence of interfering substances. No further attempts were made to separate this portion, since this fraction probably resulted from the solubility of the Neuberg precipitate.

(*e*) *Neuberg Precipitate.* No figures are given for nitrogen contents of fractions V and VII, since total nitrogen determinations on these fractions indicated less nitrogen than was subsequently recovered. The nitrogen accounted for after fraction V would indicate that its nitrogen content was 287 mgm. N; the loss of 90 mgm. being the sum of the losses in subsequent operations.

The mercury-free solution (V), was adjusted to pH 7 and extracted for 60 hours with butyl alcohol under reduced pressure (6).

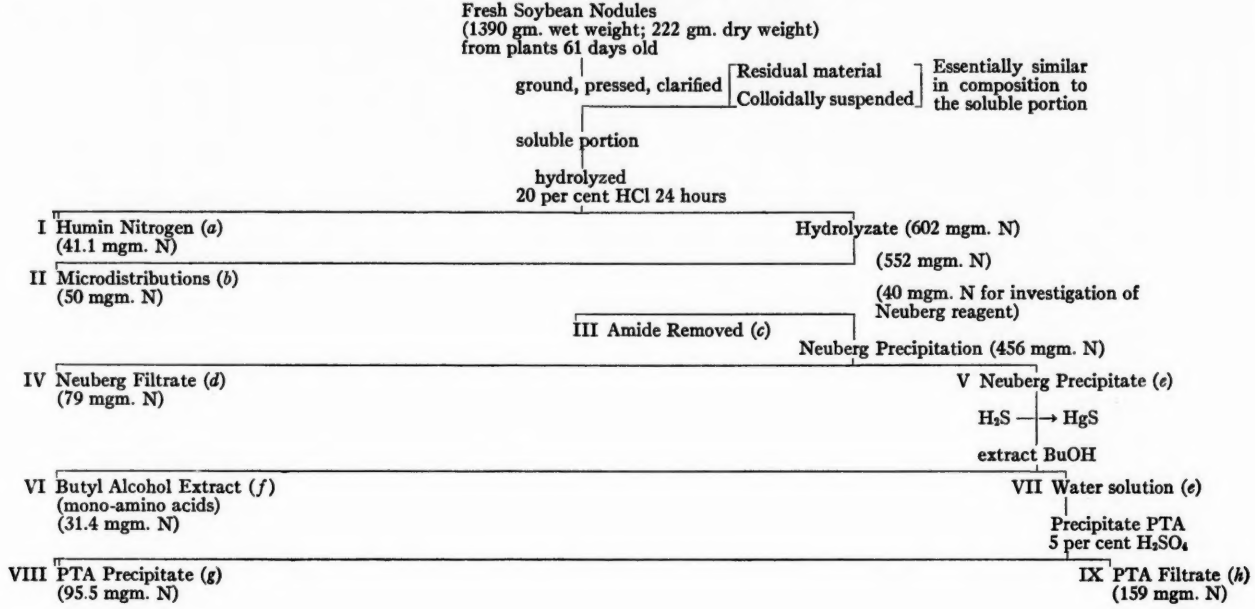
(*f*) *Mono-amino Acids (Butyl Alcohol Extract).* After removal of BuOH this fraction was subjected to fractionation by copper salts (3, 22), yielding the following results:

	Per cent of fraction
Water insoluble—leucine, phenylalanine, norleucine (?) and methionine (?)	4.4
Water soluble—	
MeOH insoluble—glycine, alanine, serine, tyrosine	14.4
MeOH soluble—valine, isoleucine, proline, hydroxyproline	81.2

Apparently the greater share of the amino acids present is soluble both in water and in methyl alcohol.

TABLE 5

Scheme of analysis of the soluble portion of fresh nodular tissue



(*g*) *Basic Fraction* (*PTA Precipitate*). The PTA precipitate was made just alkaline to phenolphthalein, $Ba(OH)_2$ added to complete precipitation, Ba removed by H_2SO_4 and analyzed by the methods outlined by Cavett (4). Aeration of an alkaline aliquot showed that 63.1 per cent of the nitrogen could be liberated as NH_3. Inasmuch as ammonia and amide nitrogen had been removed (step III, note *c*), no NH_3 should be present in this fraction unless decomposition of easily hydrolyzable nitrogen compounds had taken place. Arginine was determined by digestion and distillation with strong alkali. The results were somewhat variable but showed that arginine comprised about 21.4 per cent of the basic fraction. It has been accepted as preferable to determine arginine directly on the acid hydrolyzate before separation with PTA. Such a determination on a portion of the amide- and NH_3-free hydrolyzate showed 88 per cent of the basic fraction as arginine nitrogen. These figures indicate that a large portion of the arginine ($\frac{63}{88}$ = 72 per cent) was apparently decomposed during the removal of the PTA and the subsequent aeration for ammonia.

Histidine comprised 9.2 per cent of the fraction as determined by the diazo reaction outlined for microapplication by Cavett (4). The Kapeller-Adler method (13) gave no color reaction, though a color was obtained on adding histidine to the solution. The latter method is presumably more specific and renders the results of the diazo reaction somewhat questionable; however, the concentration of histidine approached the limit which is detected by the Kapeller-Adler method.

Cystine comprised 0.67 per cent of the basic fraction as determined by the uric acid reagent method. This is not in agreement with the Zahnd and Clarke determination (30) of cystine by alkaline hydrolysis (in the presence of lead acetate) of the original plant sap, which indicated 1.48 per cent of the total nitrogen as cystine. The discrepancy may be explained by the destruction of cystine which is somewhat sensitive to acid hydrolysis and by the incomplete precipitation of cystine by PTA reagent (7).

The composition of the basic fraction was found to be as follows:

		Per cent	
Arginine	as NH_3	63.1	88 per cent as determined (as arginine) directly on hydrolyzate
	on alkaline digestion	21.4	
Cystine		0.7	
Histidine		9.2	
Lysine (by difference)		5.6	

Unfortunately, insufficient material was available to determine lysine as the picrate. It can hardly be accepted as satisfactory to consider lysine as the difference from 100 per cent recovery, as the unspecific nature of the color reactions employed give but an approximation of the true amino acid content in complex mixtures.

From the results obtained by the arginine determination directly on the acid hydrolyzate, it would appear that this amino acid, or some other organic nitrogen compound which liberates nitrogen on digestion with alkali, was largely decomposed in subsequent treatments. Apparently the employment of aeration in alkaline solutions, which has proved satisfactory for general use with plant saps (18), can not be applied to the isolated basic fraction, possibly because of the low buffering capacity of the separated fraction. Of chief interest is confirmation of the observation made on the dried nodular tissue that arginine (compounds liberating NH_3 on strong alkaline hydrolysis) comprises a large portion of the basic fraction. Since arginine has an extremely high ratio of nonamino to amino nitrogen (3/1), it seems probable from these data, as well as from those on the dried tissue, that this amino acid may contribute largely to the basic nonamino nitrogen, implicated in the nitrogen fixation process by Orcutt (16).

Apparently lysine is very low in nodular tissue, comprising only 3 to 6 per cent of the basic fraction.

(*h*) *PTA Filtrate.* This fraction contained a large quantity of inorganic salts, and attempts made to separate dicarboxylic acids as their Ca or Ba salts were unsatisfactory.

The data are summarized in table 6; as the figures were calculated on the same basis as those of table 4, the remarks regarding the significance of the actual values apply here as well.

In summary the following conclusions may be noted:

The soluble portion of the fresh tissue differs little, if at all, from the entire dried tissue. Possibly the mono-amino acid (BuOH extract) fraction comprises a lower proportion of the nitrogen but because a different procedure was used in isolation of this fraction from the soluble forms of nitrogen (precipitation with Neuberg reagent), the difference indicated by

TABLE 6

Summary of analysis on soluble portion of fresh soybean nodules

FRACTION	AS PER CENT OF FRACTION	AS PER CENT OF TOTAL N
Humin-N	.....	4.3*
Amide + NH_3	.....	11.5*
Basic-N	.....	28.8*
Arginine†	84.5	24.3
Histidine	9.2	2.7
Cystine	0.7	0.2
Lysine	5.6	1.6
Nonbasic-N	.....	54.7*
Neuberg filtrate	17.3*	9.5
Alcohol-soluble	52.0	4.9
Alcohol-insoluble	48.0	4.6
BuOH extract	13.6	7.4
Cu salts—water-insoluble	4.4	0.3
Cu salts—MeOH-insoluble	14.4	1.1
Cu salts—MeOH-soluble	81.2	6.0
Non-extracted	69.1	37.8

* From microdistribution data (note *b* to table 5).

† See note *g* to table 5.

the data in table 6 may not be so great as it appears. Of the total nonbasic nitrogen, 17.3 per cent was found in the Neuberg filtrate, and its composition was undetermined. This possibly could have caused the figures for the butyl-alcohol-soluble nitrogen to be unduly low.

As with the dried tissue, arginine comprises a large portion of the basic fraction. It seems likely that the nonamino basic nitrogen, implicated in the symbiotic nitrogen fixation process by Orcutt (16), is primarily composed of arginine.

SUMMARY

Analyses of the nitrogenous components are presented for four species of the root nodule bacteria. These analyses show that, although certain minor differences exist among the species examined, the composition of the species in general is very similar and is characterized by a rather low content of basic nitrogen.

Previous studies on the chemical composition of the root nodules of the soybean, particularly in relation to the symbiotic nitrogen fixation process, had given rise to inconsistent and incomplete data, necessitating further analysis, which are reported in this paper. The conclusions, discussed more fully in the text, may be briefly summarized as follows:

In a comparison of the nitrogen fractions of different portions of the soybean plant, the data indicate that both the entire fresh tissue and its soluble portion are alike throughout the plant. No unique components exist in the nodule, with the possible exception of nonamino basic nitrogen. It appears that the difference in composition of the nodules relative to the remainder of the plant which has been emphasized by other investigators is primarily of a quantitative rather than of a qualitative nature; that is, nodules are higher in total nitrogen per unit weight, but, in general, the same types of organic nitrogen compounds are found in these specialized tissues as in the other portions of the plant.

In a detailed analysis of *dried* nodular tissue, arginine (basic substances which yield NH_3 on alkaline digestion) was observed to constitute almost 20 per cent of the nitrogen in the tissue. It was further shown that the nonamino nonbasic ("other") nitrogen fraction, which has been found in increasing proportion in plants as they mature, is probably proline.

In a detailed analysis of the *soluble* portion of fresh nodular tissue it was likewise found that arginine comprises a very large percentage of the basic amino acids present. It seems likely that the basic nonamino nitrogen previously suggested as of possible importance in the symbiotic nitrogen fixation process originates from the arginine content of the basic fraction.

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REDUCTION OF SOIL POPULATIONS OF THE ROOT-KNOT NEMATODE DURING DECOMPOSITION OF ORGANIC MATTER[1]

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The effect of decomposition of green manures and of other organic additions on the general soil microbiological population is widely recognized as profound, yet its effect on soil populations of plant-parasitic nematodes seems not to have been examined closely. Various types of free-living nematodes are relatively abundant in soils rich in organic matter and in decomposing plant wastes, where they feed upon decomposing organic matter itself (saprophagous) or upon soil microorganisms (microphagous and fungus-sucking). An increase in abundance of unidentified nematodes has been observed to follow decomposition of cellulose in soil (11). No adequate experimental study of the relationship of decomposition to populations of nematodes in general, or of any specific nematode, appears to have been recorded.

Scattered notes appear in the literature on the effects of green manures and organic mulches on plant-parasitic nematodes. For minimizing root-knot nematode injury to orchard trees, Watson (20) recommended the use of deep mulches of straw, weeds, or other materials. Fawcett (8), discussing the variable severity of infestations of the citrus-root nematode, *Tylenchulus semipenetrans* Cobb, 1913, suggested the possibility that parasites of the nematode were a factor, and said: "Under California conditions one important factor appears to have consisted in the use of suitable amounts of bulky organic fertilizers." Thorne (19), dealing with the sugar-beet nematode, *Heterodera schachtii* Schmidt, 1871, stated: "When a heavy crop [of sweet clover] is plowed under, the heat and gases of the decaying material kill large numbers of the nematodes, including those within the brown cysts. Examination of soil from such fields showed that the numbers killed varied from about 5 to as high as 22 per cent of the nematodes."

Stewart *et al.* (18) found fewer nematodes of three species in roots of sugar cane grown in soil treated with molasses, "mud press," or compost than in check soil or soil that received only inorganic additions and found that the organic materials improved top and root growth. They also found, in one

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soil, that addition of molasses was followed by an increased population of *Mononchus*, a genus of predacious nematodes.

An association of microorganisms engaged in decomposition not only might, in some manner, render the soil environment deleterious to the survival of plant-parasitic nematodes, but might also alter the activity or abundance of known natural enemies of nematodes in soil. Among these are predacious nematodes, including *Mononchus* Bastian, 1865, *Dorylaimus* Dujardin, 1845, and allied genera (14), and certain *Aphelenchoides* Fischer, 1894, (13, 14); predacious mites (10); and numerous fungi, including forms which capture nematodes with highly specialized hyphae (3, 4, 5, 6, 7, 23), as well as non-trapping parasites (3, 16, 22).

Experiments begun in October, 1935, to determine the influence of decomposing organic matter in soil naturally infested with the root-knot nematode, *Heterodera marioni* (Cornu) Goodey, 1932, have been reported briefly in a preliminary note (12), which showed a stimulated activity of natural enemies of nematodes to account for striking reductions in populations of infective larvae. This paper reports experimental methods, gives additional results, and partially interprets the phenomena involved.

MATERIALS AND METHODS

Soil for each of five experiments was collected immediately prior to use from areas heavily infested with *Heterodera marioni* within two ratoon pineapple fields on the island of Oahu, now known to contain several biological control factors. Sufficient soil for an experiment, taken from the surface 6 inches, was mixed as one batch, passed through a screen (4 or 8 meshes per linear inch), then weighed in 2,400-gm. quantities (moist weight) into wide-mouthed gallon glass jars. No additional water was required at this time. Organic matter was added by weight and mixed in thoroughly by rotating and shaking the jars. During decomposition the mouth of the jar was covered by only one thickness of muslin to allow free exchange of gases.

Pineapple plants were freshly collected for each experiment from the same source as that of the soil to prevent introduction of biological factors foreign to the particular locality, and a sufficient weight of entire plants was chopped and mixed as one sample to ensure a representative proportion of dead leaves and of young and old live tissue. Except where noted, chopping reduced solid pieces to approximately 2 cm. in greatest dimension.

During decomposition, all the soil jars of one experiment were kept in one laboratory out of direct sunlight. At specified intervals, check and decomposition jars were shaken and rotated to mix their contents, weighed, and watered to restore to their initial weights. In the first two experiments, temperatures were read daily from the glass-stem thermometers, the bulbs of which were at the center of the soil mass in duplicate jars of each treatment, but this practice was discontinued after it was found that heating during decomposition was slight and of short duration (tables 1 and 3).

When the allotted decomposition period had passed, soil from each jar, after being thoroughly mixed, was divided equally among four pots (no. 2½ canner's tins) and planted with seeds of the Whippoorwill variety of cowpea (*Vigna sinensis* Endl.), 3 seeds per pot in experiments I, II, and III, and 5 per pot in experiments IV and V. The cowpeas served as indicator plants (9), their roots being washed and the nematode galls counted after 30 to 35 days.

Tests of statistical significance of differences between treatments are based on the standard error of difference, following Miles' table (15), with standard errors of treatment means calculated from the total galls per jar of 2,400 gm. soil (sum of sums of galls in the four pots planted from one jar).

Soil samples, taken at the time of planting indicator cowpeas, were tested for nematodes by a semi-quantitative technic. The nematodes were washed out and concentrated, by a form of the Cobb technic involving decantation and

TABLE 1

Heterodera galls developed on cowpea roots and various nematodes separated from soil after decomposition of three forms of organic matter—experiment I

Duration of decomposition, 12 weeks; interval between mixings, 7 days

ADDITIONS	MAXIMUM TEMPERATURE	GALLS PER JAR OF 2,400 GM. SOIL						NEMATODES PER 10 GM. SOIL	
		1	2	3	4	Mean	t† from check	Total	Dorylaims
	°C.								
Water, 50 cc.	26.5	712	658	501	572	611.0 ±46.51	.....	7.5	1.2
Sugar, 100 gm.‡	31.2	...*	.....	.....	.....	.....	.....	505.0	0
Panicum, 330 gm.	27.6	15	22	9	20	16.5 ± 2.90	12.76	173.8	2.5
Pineapple, 400 gm.	27.9	0	2	4	2	2.0 ± 0.82	13.09	71.8	20.0

* Cowpea indicator plants failed to grow.

† t = 5.2 required for odds 999:1.

‡ Plus 100 cc. water.

screening, and then were counted, in convenient taxonomic groups, under a microscope. Numbers of total nematodes and numbers of predacious dorylaims[2] per 10 gm. soil plus organic residues are tabulated for each experiment along with the gall count data.

EXPERIMENTAL

Experiment I

The initial experiment involved soil containing 20.9 per cent water, with additions of tap water, cane sugar, a coarse grass (*Panicum barbinode* Trin.), and pineapple (table 1). Dry-matter equivalents of the last two were 87.6 gm. and 115.7 gm., respectively, per 2,400 gm. soil. Growth of fungi promptly appeared on the soil surface and against the glass in all decomposition jars.

[2] Throughout these studies, species of *Dorylaimus* Dujardin, 1845, and *Discolaimus* Cobb, 1913, were grouped as dorylaims.

Such growth recurred most persistently after the soil was mixed, over a long period, with pineapple, and least persistently, with sugar. After 6 weeks, oligochaete worms were abundant against the glass with pineapple, occasional with grass, and absent from the other series. Mites similarly became most numerous with pineapple. Both worms and mites then declined in abundance until at the end of 12 weeks they were rare. Nematodes, when first looked for, after 10 weeks, were abundant against the glass in the sugar series, occasional in the grass and pineapple series, and not to be seen in the check. These were chiefly free-living species rather than *Heterodera marioni.*

After 12 weeks, when the soil was transferred to pots and indicator cowpeas were planted, soil of the various series compared as follows: check, compact and close grained; sugar, somewhat more bulky and much more friable; grass, still more bulky, with much fine plant residue; and pineapple, equal to grass in bulk, but with little plant residue. Differences in absorption and retention of water after planting, favored strongly the three decomposition series.

Nematodes separated from soil samples were much more numerous in the three decomposition treatments than in the check (table 1). These were chiefly cephalobs,[3] dorylaims, *Aphelenchus avenae* Bastian, 1865, a fungus sucker (1), and *Ditylenchus intermedius* (de Man) Filipjev, 1936, a fungus sucker. Plant-parasitic forms were relatively rare.

At the same time, soil samples placed on water agar in Petri dishes yielded cultures of four species of nematode-capturing fungi from the pineapple series, three from the panicum, and none from sugar or check. Nemas were observed crawling from the soil clumps with hyphal traps attached, leaving no doubt that these fungi were capturing nematodes within the soil. No adequate tests were applied for non-trapping parasites.

Numbers of nematode galls on the cowpea roots (table 1) demonstrated a striking reduction in numbers of infective larvae in the soil in which grass and pineapple had decomposed as compared with the check soil in which starvation and the normal activities of natural enemies had left a heavy infestation. In the sugar series, cowpeas germinated but soon died.

The 16 small pots of soil per treatment in which the cowpeas had grown were emptied into 4 larger pots (no. 10 canners' tins), and pineapple slips (Cayenne variety, matched by weight) were planted. After 10 months' growth without fertilizer, the pineapple plants were removed and examined. Table 2 compares top growth and root systems; plate 1 illustrates root systems of 2 treatments.

Superiority of all decomposition treatments over the check is striking with respect to top weight increase and extent and condition of root systems. In the check series, death and decay of galled roots had obviously reduced the number of galls counted to far below the number developed, and few roots were actually growing. In the three decomposition series root decay had been

[3] Throughout these studies, various genera of the family *Cephalobidae* Chitwood and Chitwood, 1934, were counted together as cephalobs.

slight, and vigorous root growth was in progress. Here the abundance of live roots had permitted multiplication of nematodes that had survived the period of active decomposition, building up large numbers of galls, especially in the grass series. In all the decomposition treatments, however, it was evident from the positions of galls on roots, that the initial root systems were very slightly infested, for most of the galls were on roots of later development. In

TABLE 2

Pineapple plants grown 10 months from slips without fertilizer in soil following decomposition of the organic materials specified and growth of indicator cowpeas—experiment I

DECOMPOSITION TREATMENT	PLANT	SLIP WEIGHT	TOP WEIGHT	WEIGHT INCREASE	ROOT WEIGHT	LENGTH OF MAIN ROOTS	ROOTS WITH GALLS	NUMBER OF GALLS		
								On main roots	On lateral roots	Sum
		gm.	*gm.*	*per cent*	*gm.*	*cm.*	*per cent*			
Check	1	109	238	118	7.2	340	41	80	178	258
	2	109	415	281	19.5	428	83	141	336	477
	3	111	416	275	25.5	479	88	167	503	670
	4	112	350	212	12.5	300	73	81	191	272
	Mean	110	355	222	16.2	387	71	117	302	419
Sugar	1	106	510	381	31.0	753	51	35	24	59
	2	109	427	292	39.0	972	47	71	66	137
	3	110	614	458	30.5	623	16	13	87	100
	4	113	499	342	43.0	833	38	38	66	104
	Mean	109	512	368	35.9	795	38	39	61	100
Panicum	1	107	679	535	32.5	730	87	149	396	545
	2	109	484	344	22.0	710	63	125	395	520
	3	111	387	249	22.0	724	90	170	339	509
	4	113	455	303	32.5	691	81	114	317	431
	Mean	110	501	358	27.3	714	80	139	362	501
Pineapple	1	108	581	438	38.5	962	34	44	36	80
	2	109	526	383	34.0	867	81	101	207	308
	3	110	538	389	38.0	903	62	81	163	244
	4	...	...	...	....	...	..	...	...	...
	Mean	109	548	403	36.8	911	59	75	135	211

the check series, on the contrary, even the initial roots were conspicuously galled. These data indicate that decomposition of sugar reduced the population of infective larvae even more than did pineapple. Part of the growth superiority following grass and pineapple, as compared with the check, may be a consequence of release of nutrients from the decomposed plant material, but this factor is excluded in the sugar treatment.

Experiment II

Decomposition of four graded amounts[4] of chopped plant (table 3) was compared with two checks, one with water added approximately equal to that contained in 300 gm. plant material, the other with no water added. Each treatment comprised four soil jars. After 12 weeks, nematodes of the following groups were more numerous in the decomposition jars than in the checks, but were not in direct proportion to the amount of organic matter added: *Ditylenchus intermedius*, *Aphelenchoides parietinus* (Bastian) Steiner, 1932, *Rhabditis* spp., cephalobs, and dorylaims. *Rhabditis* was by far the most abundant form except in the check soil.

Gall counts on cowpea roots (table 3) indicated a progressively greater reduction of infective larvae with increasing organic additions up to 300 gm. per jar, but 400 gm. was apparently slightly less effective than 300 gm. The

TABLE 3

Heterodera galls developed on cowpea roots and nematodes separated from soil after decomposition of varied amounts of chopped pineapple plant—experiment II

Duration of decomposition, 12 weeks; interval between mixings, 7 days

ADDITIONS	MAXIMUM TEMPERATURE	GALLS PER JAR OF 2,400 GM. SOIL						NEMATODES PER 10 GM. SOIL	
		1	2	3	4	Mean	t† from dry check	Total	Dorylaims
	°C.								
0	24.7	181	230	165	184	190.0 ±13.97	.....	1	0
Water, 225 cc.*.....	24.5	99	121	102	131	113.2 ± 7.79	4.80	1	0
Pineapple, 100 gm.....	25.4	71	106	51	35	65.7 ±15.34	5.99	38	1
Pineapple, 200 gm.....	26.3	19	26	39	39	30.7 ± 5.02	10.73	35	2
Pineapple, 300 gm.....	26.7	22	28	14	19	20.7 ± 2.98	11.86	58	6
Pineapple, 400 gm.....	27.1	33	31	33	11	27.0 ± 5.35	10.90	54	7

* 234 cc. would have been equal to water in 300 gm. pineapple.

† t = 3.2 required for odds 106:1; t = 5.2 for odds 999:1.

two checks were strikingly and consistently different, indicating that the wetter soil, which was too wet for good tilth, was either directly less favorable for *H. marioni* larvae or more favorable for some of their enemies.

Experiment III

There were two variables in experiment III—frequency of mixing of the soil and fineness of chopping of the plant material (table 4)—and four soil jars for each treatment. Two check treatments without added water, one for each frequency of mixing, were maintained. Material designated "fine" was chopped until few solid pieces more than 5 mm. in diameter remained; "coarse" included leaves cut to approximately 1-inch squares and stems cut to approxi-

[4] Approximately equivalent to 50, 100, 150, and 200 tons per acre-foot of the soils dealt with.

mately 1-inch cubes. The weight added was uniformly 300 gm. After 12 weeks, total nematodes and *Aphelenchoides parietinus*, *Rhabditis* spp., cephalobs, and dorylaims were more numerous in decomposition treatments than in the checks, but differences among the four decomposition treatments were not significant.

Gall counts on cowpea roots (table 4) indicated, as in former experiments, that decomposition was accompanied by a great reduction in population of infective larvae. Effectiveness was not closely related to either frequency of mixing or fineness of chopping, but, in the checks, mixing every 7 days resulted in fewer galls than did mixing every 21 days.

TABLE 4

Heterodera galls developed on cowpea roots and various nematodes separated from soil after decomposition of finely and coarsely chopped pineapple plant with mixing every 7 and 21 days—experiment III

Duration of decomposition, 12 weeks; 300 gm. pineapple plant per jar of 2,400 gm. soil

INTERVAL BETWEEN MIXINGS	ADDITIONS	GALLS PER JAR OF 2,400 GM. SOIL							NEMATODES PER 10 GM. SOIL	
		1	2	3	4	Mean	*t** values from		Total	Dorylaims
							Check	7-day mix		
days										
7	0	1,089	1,269	836	949	1,036 ± 93.5	.....	....	3	0
7	Fine	60	90	115	96	90 ± 11.5	10.04	....	24	1
7	Coarse	175	68	131	81	114 ± 24.6	9.54	....	35	3
21	0	1,527	1,515	964	1,653	1,415 ±153.5	.....	2.11	4	0
21	Fine	106	108	59	118	98 ± 13.2	8.55	0.46	44	6
21	Coarse	145	120	113	92	118 ± 10.9	8.43	0.15	32	3

* $t = 2.0$ required for odds 20.6:1; $t = 3.2$ for odds 106:1; $t = 5.2$ for odds 999:1.

Experiment IV

Experiment IV compared the effects of 300 gm. fresh pineapple plant, chopped as in the first two experiments, with an equivalent amount of plant material from the same lot but oven-dried before addition to the soil. The dry matter content of this lot was 21.04 per cent. Accordingly, 63 gm. dried material plus 237 cc. water was taken as the equivalent of 300 gm. fresh weight. These quantities were added to 2,400 gm. soil in gallon jars, 7 jars per treatment, and decomposition was allowed to proceed for 12 weeks with mixing and watering to restore lost weight at 14-day intervals.

During early weeks of decomposition, growth of mycelium was more conspicuous with the dried material than with the fresh, indicating more rapid decomposition. Mean losses of weight per jar during the first 14 days were 67.6 gm., 98.4 gm., and 85.1 gm. for the check, dried, and fresh series, respectively. These losses, which include both water vapor and gaseous products

of decomposition, indicate more rapid decompos'tion of the dried (and killed) tissues.

Counts of nematodes washed from small samples of soil from three jars of each treatment, at the end of 12 weeks, showed the dorylaims and cephalobs to be more numerous in the decomposition treatments than in the check. *Heterodera marioni*, on the contrary, averaged 21.3 per 10 gm. in the check, compared with only 0.3 in the dried series and 1.0 in the fresh series, equivalent to 5,112, 72, and 240, respectively, per jar of 2,400 gm. soil.

Galls counted on indicator cowpea roots (table 5) indicated great reductions associated with decomposition, and greater reductions with dried than with fresh plant material. The differences between treatments in gall counts are much smaller than in direct counts of larvae, but it must be recognized that both of these measurements are merely semi-quantitative. It is very appar-

TABLE 5

Heterodera galls developed on cowpea roots and various nematodes separated from soil after decomposition of fresh vs. oven-dried chopped pineapple plants—experiment IV

Duration of decomposition, 12 weeks; interval between mixings, 14 days

ADDITIONS	GALLS PER JAR OF 2,400 GM. SOIL										NEMATODES PER 10 GM. SOIL	
	1	2	3	4	5	6	7	Mean	*t** values from Check	*t** values from Fresh	Total	Dorylaims
0	4317	5042	4554	4996	6083	5093	4402	4927 ±227.3	....	....	39	1.0
Dried, plants, 63 gm. plus 237 cc. water.	369	304	340	409	382	393	315	359 ± 15.0	20.1	6.6	47	21.0
Fresh plants, 300 gm.....	497	534	654	517	673	501	589	566 ± 27.7	19.0	....	48	21.3

* $t = 5.7$ required for odds 20,000:1.

ent, however, that so slight a variable as the desiccation of plant material may significantly alter the effectiveness of decomposition as it relates to Heterodera populations.

Experiment V

Experiment V (table 6) was carried out on a large scale to determine whether *H. marioni* populations are reduced suddenly or gradually. All decomposition treatments received 300 gm. freshly chopped plant containing 23.7 per cent dry matter. Initial soil moisture was 26.19 per cent, dry weight basis, and no water, except that contained in the plant tissue, was added to any series at the outset. Soil was mixed each 14 days when water was added to restore weight lost. Throughout, each treatment comprised six soil jars.

At the start, cowpeas were planted in check soil from six jars, to measure

the initial population of infective larvae; then, at intervals of 4 weeks, one pair of treatments (check and decomposition) was planted with cowpeas, the fifth and last pair being planted after 20 weeks. This experiment provided five comparisons of decomposition vs. check, five different decomposition treatments with elapsed time as the variable, and six checks with elapsed time as the variable. An uncontrolled variable was introduced by planting the indicator cowpeas during different seasons, the planting dates ranging from May 15 to October 2.

TABLE 6

Heterodera galls developed on cowpea roots and various nematodes separated from soil after varied periods of decomposition of chopped pineapple plant—experiment V

300 gm. pineapple plant per jar of 2,400 gm. soil; interval between mixings; 14 days

DURATION OF DECOMPOSITION	CHOPPED PINEAPPLE ADDED	GALLS PER JAR OF 2,400 GM. SOIL										NEMATODES PER 10 GM. SOIL	
									t* values from				
		1	2	3	4	5	6	Mean	0 weeks	Paired check	Prior decomposition	Total	Dorylaims
weeks													
0	−	9,401	5,904	6,272	5,271	5,220	8,562	6,772 ±725.4	...	....	...	....	....
4	−	7,875	6,328	8,576	6,729	5,604	8,773	7,314 ±525.3	0.6	....	...	23.3	2.6
4	+	626	562	432	388	335	209	425 ± 62.0	...	13.0	...	99.3	7.6
8	−	3,268	2,022	1,840	2,148	1,631	2,466	2,229 ±237.6	5.9	....	...	48.0	1.0
8	+	487	837	654	677	392	605	609 ± 63.4	...	6.6	2.1	57.3	7.0
12	−	6,495	5,070	4,344	5,982	6,068	3,833	5,299 ±431.8	1.7	....	...	10.0	1.0
12	+	987	1,027	625	827	1,781	645	982 ±173.8	...	9.3	2.0	16.6	3.6
16	−	3,042	6,012	5,159	6,941	5,095	4,801	5,175 ±532.9	1.8	....	...	50.0	0
16	+	1,078	1,067	1,433	1,280	1,212	1,333	1,234 ± 59.0	...	7.3	1.4	70.7	6.6
20	−	5,058	5,326	5,757	5,662	4,468	4,224	5,082 ±256.0	2.2	....	...	....	....
20	+	1,607	1,380	1,458	1,762	1,461	1,322	1,498 ± 65.8	...	13.6	3.0	....	....

* t = 1.9 required for odds 22:1; t = 2.8 for odds 105:1; t = 4.2 for odds, 1,110:1; and t = 5.5 for odds 9,999:1.

Nematodes washed from the soil and counted, as each treatment was prepared for the planting of indicator cowpeas, showed wide fluctuations in total nematode populations (table 6) and in abundance of different nematodes. At each time of sampling, however, the total nematode population was higher in the decomposition treatment than in the check, whereas the population of *H. marioni* was lower in the decomposition treatment than in the check. In every comparison the following nematodes were more abundant in the decomposition treatment: cephalobs, dorylaims, and *Rhabditis* spp.

The gall counts in table 6 again provide abundant proof that decomposition is accompanied by a reduction of the infective population of *H. marioni*, but

they indicate certain variations from one sample pair to another that are not readily interpretable. In the checks, it appears that only very slight reduction of the infective population occurred, from starvation and other causes, during 20 weeks. The much lower gall counts in the 8-week check than in earlier or later checks is inexplicable at present. The decomposition treatments are most surprising, for here galls were fewest after 4 weeks and were progressively more numerous with each succeeding period of 4 weeks, until, after 20 weeks, 3.5 times as many galls were present as after 4 weeks. The number was still far smaller than that in the 20-week check, but it is evident that, with increasing time, the spread between checks and decomposition treatments had become less. That external conditions, as of temperature, were not wholly responsible for this is evidenced by the simultaneous occurrence of most galls in the check and fewest in the decomposition treatment after 4 weeks.

DISCUSSION

The results of these experiments demonstrate that decomposition of organic matter, in the soils dealt with, is accompanied by marked changes in nematode populations. Some common free-living nematodes, including *Rhabditis* spp., cephalobs, and dorylaims, multiply and remain more numerous than in check soils over at least 4 months, favored by an abundant food supply. Members of the first two groups feed upon decomposing matter itself or upon microorganisms, whereas the dorylaims feed as predators upon other nematodes, rotifers, and other soil microfauna. Populations of *Heterodera marioni*, on the contrary, decline under these conditions. This obligate plant parasite multiplies only in the roots of living plants, and these were absent during decomposition in these experiments. In all experiments, populations of this parasite were compared with checks in which, similarly, there had been no opportunity for multiplication. Conditions of the experiments excluded desiccation and irradiation. It appears, therefore, that some consequence of the decomposition must account for destruction of *H. marioni* in these experiments, and this appears to have been a stimulated activity of natural enemies, both fungi and predacious animals.

Evidence presented in the earlier note (12) previously mentioned points to fungi as of major importance. Further evidence, from detailed studies of nematode population changes at shorter intervals, will appear in a following paper. Of these fungi, the several nematode-trapping forms with highly specialized hyphae appear more important, under these conditions, than the non-trapping parasites. Nematode-trapping fungi are widely established in Hawaiian soils. Most of them grow readily in pure culture on ordinary media, but they appear relatively ineffective, in competition with other soil organisms, as agents of decomposition of plant substance. Given a sufficient population of live nematodes, however, they thrive, for they then find a source of food unavailable to other types of fungi.

Predacious animals, both nematodes and mites, also require consideration.

At the time the earlier note (12) was written, predacious nematodes appeared of no importance, as no species of *Mononchus* was encountered. Since that time, however, it has been learned that the dominant species of *Dorylaimus* and *Discolaimus* in certain Hawaiian soils are predacious on other nematodes (14). These dorylaims were found consistently, in these experiments, to multiply in soil to which pineapple material was added. Predacious species of *Aphelenchoides* (14) have likewise been recognized since these experiments were concluded and have been found in both localities from which soil was obtained for these experiments. What part they played in determining results is not known. No attempt was made to estimate the significance of mites of various kinds which were abundant in the decomposition treatments. In an experiment now in progress, however, the dominant species of mite (undetermined) which rapidly builds up large populations during decomposition, is a facultative predator, devouring nematodes freely. The relative importance of these several factors cannot be estimated from available data, but the evidence is definite that enemies of nematodes, both plant and animal, are favored by decomposition.

The abundant food supply for saprophagous and microphagous nematodes, made available by decomposition, leads to rapid multiplication and the building up of large populations. These nematodes then feed the nema-destroying fungi and predacious animals, which consequently multiply or increase in activity until the saprophagous and microphagous forms, destroyed more rapidly than they can multiply, decline in numbers. At the same time, many of the larvae of *Heterodera marioni*, unable to multiply, are killed by the predacious animals and by those fungi which are not specifically limited to other forms of nematodes.

That rapidity of decomposition may be highly important is indicated by experiment V (table 6), in which the maximum reduction of gall numbers was recorded after 4 weeks, and by the greater effectiveness of oven-dried material in experiment IV. Other data mentioned earlier (12), which later will be reported in full, indicate that nematode populations may rise and fall very rapidly after decomposition of organic matter begins. If we are dealing chiefly with a stimulated activity of natural enemies, then the extent of reduction of *H. marioni* populations may be expected to depend upon the intensity of the total nematode population built up at the outset and the resultant intensity of activity of biological control factors.

The progressive rise in numbers of galls on cowpea roots after the initial 4 weeks, in experiment V, demonstrates the operation of some factor beyond mere destruction of *H. marioni* larvae. If this nematode is truly an obligate parasite, then something associated with decomposition either made the indicator cowpea roots relatively resistant to its attack, or reduced the tendency of this nematode to enter roots, or delayed the hatching of its eggs. It may be significant that cowpea growth was distinctly superior in the decomposition treatments not only of this experiment but of other experiments, as indicated

by greater top weight and by greater diameter of rootlets. Associated with these differences, the roots may have been resistant to invasion, or, on the contrary, these differences may have resulted from relative freedom from root infestation.

The practical significance of these results as applied to agriculture is not determined. No prospect of eradication of the root-knot nematode is afforded, and nothing less than eradication is sufficient for some purposes, as in nurseries and propagating beds. For the growth of some crops, however, even a slight reduction of Heterodera populations may be helpful. The degree of reduction obtained here, though always very highly significant statistically, varied widely between experiments, and until more is known of what caused these variations it is impossible to judge whether higher effectiveness may be obtainable. These findings, together with the beneficial results in nematode control formerly attributed by other investigators to the addition of organic matter to soil, suggest that further stress might well be laid on the use of organic mulches and green manures where obligate plant-parasitic nematodes are troublesome. It must be recognized, however, that certain free-living nematodes may act as facultative parasites. *Aphelenchoides parietinus*, which multiplied in one of these experiments and thrives in colonies of fungi, also damages cotton seedlings (1). Another, *Aphelenchus avenae*, is known to invade living plants (17). *Aphelenchoides fragariae* (Ritzema Bos) Christie, 1932, which damages strawberry, also feeds upon fungi (2) and might increase during decomposition. *Rhabditis* spp. have been associated with damping-off of coniferous seedlings (21). Various other saprophagous, microphagous, and fungus-sucking nematodes have been observed by different investigators within the tissues of cultivated plants. Consequently, it may be dangerous to advocate the use of organic matter for control of obligate plant-parasitic nematodes generally, without local testing with the economic plant in question.

SUMMARY

Consistently, in five experiments, decomposition of large amounts of organic matter in soil was associated with reductions in numbers of *Heterodera marioni* galls on roots of indicator cowpeas. Reductions varied in degree but were always very highly significant statistically.

Chopped pineapple plants were the organic addition in all experiments. In the first experiment, a coarse grass and cane sugar, tested in parallel with the pineapple, gave a comparable response.

Additions of pineapple plants approximately equivalent to 50 tons, 100 tons, and 150 tons per acre-foot of soil gave progressively greater reductions in numbers of galls, but 200 tons proved no more effective than 150 tons per acre.

Varying the fineness of chopping of plants and the frequency of mixing of the decomposing material in soil had little influence upon nematodes, but oven-drying followed by wetting increased effectiveness of the organic matter.

When indicator plants were planted after 1, 2, 3, 4, and 5 months of decom-

position, the fewest galls developed after the shortest period, and the most, after 5 months, indicating that the population of Heterodera larvae is reduced early and also demonstrating the action, during decomposition, of some factor other than death of larvae.

Consistently, decomposition was followed by increases in populations of saprophagous and microphagous free-living nematodes and of predacious nematodes (dorylaims). Fungus-sucking species increased in some experiments.

The hypothesis is substantiated that decomposition results in a greatly increased population of total nematodes in the soil and that these, in turn, support the building up of large populations of plant and animal forms destructive to nematodes, including nema-capturing fungi, non-trapping fungal parasites, predacious nematodes, and predacious mites. These, collectively, attacking various types of nematodes, destroy larvae of the root-knot nematode as well as the free-living forms which comprise the greater part of the total nematode population in the soil during early weeks of decomposition.

Pineapples planted following decomposition and the growth of indicator cowpeas, in one experiment, grew much better than in check soil, apparently as a result of reduced nematode injury. With one exception, cowpea growth was superior in soil following decomposition.

Possible dangers in the practical application of these results are pointed out, showing the need for local testing with various economic plants.

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PLATE 1

PINEAPPLE ROOT SYSTEMS GROWN 10 MONTHS WITHOUT FERTILIZER IN SOIL FROM EXPERIMENT I AFTER REMOVAL OF INDICATOR COWPEAS (PHOTOGRAPHED TO SAME SCALE)

FIG. 1. Check soil, plant 3.

FIG. 2. Pineapple decomposition soil, plant 1.

Fig.1

Fig.2

ORGANIC RESIDUES AND NITROGEN FERTILIZERS IN RELATION TO THE PRODUCTIVITY AND HUMUS CONTENT OF PALOUSE SILT LOAM[1]

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The results of the early investigations of Sievers and Holtz (3, 4) on the nitrogen and organic matter relationships in the silt loam soils of eastern Washington indicate that in the course of 39 years of cropping with small grains, principally wheat alternated every second or third year by summer-fallowing, these soils lost approximately 22 per cent of their nitrogen and about 34 per cent of their humus. It was observed also that the loss of humus resulted in a pronounced deterioration of the physical condition of the soil and severe erosion of the steeper slopes during periods of heavy rains.

In a series of preliminary studies numerous laboratory experiments were conducted to determine the relation between the rate of decomposition of various organic residues added to the soil and the rate of nitrate-nitrogen accumulation in an attempt to find practical means of maintaining the soil humus supply as well as the soil productivity. The results of these experiments served as a basis for establishing field plots for the purpose of ascertaining the effect of regular applications of definite amounts of various organic residues and nitrogen fertilizers on soil fertility and soil carbon-nitrogen relationships. The data obtained from these plots previous to the year 1930 were assembled for publication by the senior author, but the completion of the manuscript was interrupted by his death on April 20, 1931. The original incompleted manuscript was revised and completed by the junior author on the basis of the additional field data obtained up to and including 1934 and the analytical results of soil samples taken in the fall of 1934.

EXPERIMENTAL PROCEDURE

For several years laboratory experiments were conducted with 1-kgm. portions of soil mixed thoroughly with different finely ground crop residues

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[2] Late associate in soils and professor of soils respectively. Grateful acknowledgment is made of the valuable assistance by L. C. Wheeting, associate in soils, and by G. O. Baker and G. M. Horner, formerly instructors in soils, in obtaining the field data since 1931, and also of the assistance by J. H. Sackett, graduate assistant, in making chemical analyses.

alone or combined with nitrogen fertilizers. The treated soils were placed in 2½-liter bottles, maintained at the normal field moisture capacity by the addition of water when necessary, and kept for 100 to 150 days at a temperature fluctuating between 20 and 25°C. The CO_2 evolved from each soil was collected in a sodium hydroxide solution and determined by titration with a standard hydrochloric acid solution after precipitation of the excess alkali with barium chloride. These determinations were made at intervals ranging from 1 day at the beginning of the experiments to 2 weeks at the end, the time interval depending upon the rate of CO_2 evolution. Nitrate-nitrogen was determined by the phenoldisulfonic acid method (1) on extracts of 1:5 soil: water mixtures at the beginning and at the end of the experiments.

The field experiments based on the results of the laboratory studies were begun in the fall of 1921 for the purpose of ascertaining the effect of repeated treatments with various organic residues and nitrogen fertilizers on crop yields and the carbon-nitrogen relationships in the soil. Three series of 0.0372-acre plots, measuring 101.3 feet in length from east to west and 16 feet in width from north to south, were laid out in an experimental field known as Field 3. The slope of this field is about 10 per cent from north to south and about 7 per cent from west to east. Nine plots separated from one another by alleys 4 feet wide comprise each of the series, which have roadways 16 feet wide between them. The plots receive regular applications of different organic residues and nitrogen fertilizers alone and in combinations and are cropped to winter wheat.

The yields of grain of the entire plots and the nitrogen content of the grain have been determined since 1921, whereas separate yields of straw and its nitrogen content have been determined only since 1932.

At the beginning of the experiments, in the fall of 1921, soil samples of the surface 6 inches and of the second 6-inch layer were obtained and kept in storage for later analysis. Twelve auger borings made at intervals of 17 feet lengthwise of each plot and at a distance of 5 feet from each side were composited for each soil sample. An identical sampling procedure was followed in the fall of 1934.

The Kjeldahl method (1) was used for the nitrogen determinations on soil and plant materials and the wet combustion method (2) for the determination of the carbon content of the soil samples and organic residue materials.

EXPERIMENTAL RESULTS

Carbon dioxide evolution and nitrate-nitrogen accumulation in treated Palouse silt loam

The data given in table 1 on CO_2 evolution and nitrate-nitrogen accumulation are typical of those obtained by various investigators from numerous laboratory experiments with soils treated with different crop residues alone or in combination with nitrogen fertilizers. They corroborate the results of a number of similar experiments reported by Sievers and Holtz (4), who con-

tended that high nitrogen accumulation is not necessarily correlated with high CO_2 evolution. The addition of straw alone to the soil as shown in table 1 caused great stimulation of CO_2 evolution and a marked depression in nitrate-nitrogen accumulation, which was not overcome after 146 days of optimum conditions for decomposition. The addition of an equal quantity of alfalfa hay or of straw supplemented with sodium nitrate did not cause any appreciably greater liberation of CO_2 than did the straw alone. The alfalfa hay, however, resulted in the accumulation of a large amount of nitrate nitrogen, whereas the straw caused an apparent depression in nitrate-nitrogen, as indicated by the figures in the last column of table 1, which show a loss of 4.2 parts per million for this treatment. The observed increase in CO_2 production obviously is a function of the organic residues, but the marked differences in nitrate-nitrogen accumulation in the soil bear a relationship to the nitrogen content of the residues or to the quantities of nitrogen supplied with them,

TABLE 1

Effect of various organic residues and nitrogen fertilizer on CO_2 evolution and NO_3—N accumulation in 1 kgm. of Palouse silt loam in 146 days

NUMBER	TREATMENT	AMOUNT ADDED	N ADDED	C EVOLVED	NO_3—N		
					Beginning	End	Gain or loss
		gm.	*gm.*	*gm.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	Check	0.00	0.0000	0.120	9.7	25.1	15.4
2	Wheat straw	2.00	0.0100	0.444	9.7	5.5	−4.2
3	Alfalfa hay	2.00	0.0512	0.446	9.7	36.9	27.2
4	$NaNO_3$	0.31	0.0510	0.104	60.7	79.5	18.8
5	Wheat straw + $NaNO_3$	2.00 0.25	0.0100 0.0410	0.427	50.7	45.3	−5.4

because the additions of nitrate nitrogen to the soil in the form of sodium nitrate alone had no appreciable effect on either CO_2 evolution or nitrate-nitrogen accumulation provided the added nitrate is accounted for.

Nitrate nitrogen did not accumulate in the soil treated with straw alone, because the amount of nitrogen made available during the process of decomposition of the straw was not in excess of the quantity needed by the active decay microbes. The apparent reduction in nitrate-nitrogen in the soil receiving straw supplemented with sodium nitrate is explainable on the same basis. Undoubtedly, a large part of the sodium nitrate added as a supplement to the straw was used up by the soil microbes during the early states of decomposition of the straw. A comparison of the amount of nitrate-nitrogen present in this soil with that in the alfalfa-treated soil at the end of the experiment indicates that an actual accumulation of nitrate-nitrogen had taken place rather than the apparent loss of 5.4 parts per million shown in the last column.

The relationship between CO_2 evolution and nitrate-nitrogen accumulation shown by the data in table 1 has an important bearing on the function of nitrogen in the transformation of organic residues to soil humus. Sievers and Holtz (3) pointed out that since the carbon-nitrogen ratio of humus in the eastern Washington soils ranges from about 9 to 13, plant residues returned to these soils do not become a part of the soil humus until the loss of carbon by decomposition has been sufficient to make the resulting product approach these ratios. On the basis of this assumption the nitrate nitrogen accumulating in the soil is largely a by-product of humus decomposition. Thus if all the carbon evolved as CO_2 during that stage of decomposition of organic residues in which the amount of nitrogen consumed by the soil microbes exceeds the amount liberated by them cannot become a part of soil humus, then as a corollary to the results indicated in table 1, organic residues with a high nitrogen content such as alfalfa hay or straw supplemented with soluble nitrogen should produce more humus than organic residues with a low nitrogen content such as wheat straw. This hypothesis was put to a test in the field experiment discussed in the following sections.

Effect of different soil treatments on crop yields

The three series of field plots mentioned earlier are numbered 100, 200, and 300 respectively and have received the following treatments regularly on the acre basis:

Plots	*Treatments*	*Pounds per acre*
100, 200, 300	Wheat straw	2,700
	Sodium nitrate	370
101, 201, 301	Alfalfa hay	2,700
102, 202, 302	Wheat straw	1,350
	Alfalfa hay	1,350
103, 203, 303	Wheat straw	2,700
104, 204, 304	Check	
105, 205, 305	Wheat straw	2,700
	Sodium nitrate	370
106, 206, 306	Sodium nitrate	370
107, 207, 307	Wheat straw	2,700
	Ammonium sulfate	286
108, 208, 308	Farm manure	12,000

Winter wheat is grown annually on the 100 series, and winter wheat is alternated by summer-fallow on the 200 and 300 series, which receive identical treatments. The crops on the 200 series are harvested in even years and those on the 300 series in odd years. The organic residues and fertilizers are applied annually in the fall before plowing on the 100 series, and in the spring before plowing for summer-fallow on the 200 and 300 series, except on plots 100, 200, and 300 where the sodium nitrate is broadcast separately early in the spring. The alleys and roadways are cultivated and seeded together with the plots in order to eliminate border effects, and the entire plots are harvested to determine yields.

Three different varieties of wheat were grown during the experimental period. Hybrid 143 was used from 1922 to 1925 inclusive, Albit from 1926 to 1930 inclusive, and Hybrid 128 from 1931 to 1934 inclusive. The data on yields calculated on the acre basis are recorded in table 2, and, as may be noted, the yields varied considerably from year to year regardless of soil treatment, variety of wheat grown, or method of cropping. The fluctuation in yields of individual plots in the 200 and 300 series under the alternate summer-fallow and wheat system of cropping is largely attributable to annual variations in climatic conditions, since for this system of cropping the supply of soil moisture is adequate to produce large yields. The yields on the plots in the 100 series, however, were affected to some degree by the available supply of moisture, as in all the years when they were high the soil in the entire root zone was saturated with moisture at field capacity at the beginning of the growing season.

In spite of the relatively low annual yields on the plots in the 100 series, the total production of grain per plot over a period of 13 years was greater, except in two cases, than that obtained by the alternate summer-fallow and wheat system of cropping. The two exceptions are the untreated and the straw-treated plots. Under the alternate summer-fallow and wheat system of cropping the addition of the various organic residues and nitrogen fertilizers alone or in combinations had no significant effect on yields, showing that with this system of farming under the prevailing climatic conditions the soil is still capable of producing maximum yields of wheat without the addition of fertilizer materials. The same treatments had a different effect in the 100 series where winter wheat was grown continuously. The addition of nitrogen in the form of organic residues or nitrogen fertilizers applied alone or in combinations resulted in substantial average yield increases, as may be noted from the data in table 2 and also from that in figure 1, which presents the averages for periods of 4 and 5 years. The increases in yields on all plots were roughly proportional to the amounts of nitrogen applied to the soil, regardless of the form in which it was added, except in the plot receiving straw alone and in that treated with manure. The straw alone, which supplied very little nitrogen, not only failed to result in increased yields but caused a slight reduction. The manure, although supplying as much nitrogen to plot 108 as did the materials added to plots 100, 101, 105, 106, and 107, was less effective than these materials in raising the yields.

An important feature shown distinctly in figure 1 is that regardless of soil treatments the 4- and 5-year average yields on the plots of the 100 series declined gradually as time progressed, although more nitrogen was added by means of organic residues and nitrogen fertilizers to all except the check and straw treated plots than was assimilated by the crop. The exact figures are given in table 4. Obviously, continuous cropping with wheat resulted in progressively diminishing yields regardless of maintenance of an adequate available supply of the principal plant nutrients in the soil. The apparent

TABLE 2

Yields of wheat in bushels per acre

PLOT NUMBER	TREATMENT	YEARS													TOTAL	AVERAGE
		1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934		
100	Straw + $NaNO_3$*	21.4	44.1	20.5	33.1	38.2	10.3	47.9	10.8	27.3	21.9	27.2	8.6	47.1	358.4	27.57
101	Alfalfa	26.3	46.1	26.1	34.9	35.1	9.9	36.1	19.2	17.9	19.6	29.1	17.2	41.7	359.2	27.63
102	Alfalfa + straw	26.5	42.7	23.2	25.0	27.9	7.1	33.6	18.2	15.7	16.4	18.9	9.4	32.3	296.9	22.84
103	Straw	29.0	35.6	17.5	25.2	21.2	7.2	26.9	15.8	10.9	12.8	15.0	4.2	26.4	247.7	19.05
104	Check	30.6	37.3	18.7	27.5	22.5	10.3	30.4	10.1	11.2	15.6	19.2	5.2	29.6	268.2	20.63
105	Straw + $NaNO_3$†	30.4	39.8	29.9	38.9	42.1	20.2	57.4	17.6	22.6	13.8	36.6	10.8	44.8	404.9	31.15
106	$NaNO_3$	33.0	45.0	23.8	34.6	27.5	23.8	62.3	15.3	19.6	10.5	32.2	4.4	42.6	374.6	28.82
107	Straw + $(NH_4)_2SO_4$†	29.2	35.8	30.1	34.9	44.1	18.9	46.0	13.9	22.7	27.6	31.5	7.3	43.5	385.5	29.65
108	Manure	26.5	27.5	31.5	31.7	35.1	14.8	39.8	18.4	18.9	29.5	21.9	7.8	42.1	345.5	26.58
200	Straw + $NaNO_3$*	20.7		36.4		44.8		55.6		44.8		35.0		26.9	264.2	37.73
300			65.7		50.2		29.9		48.1		31.0		29.8		254.7	42.45
201	Alfalfa	21.8		38.9		47.0		57.8		50.3		32.6		30.9	279.3	39.90
301			67.9		52.0		36.7		43.7		37.5		34.2		272.0	45.33
202	Alfalfa + straw	18.2		41.1		47.3		59.8		49.9		31.5		34.5	282.3	40.33
302			61.6		46.1		30.8		44.1		35.9		36.5		255.0	42.50
203	Straw	15.1		35.8		44.1		58.0		47.2		32.9		34.1	267.2	38.17
303			64.6		47.0		28.1		41.9		41.7		36.8		260.1	43.35
204	Check	25.4		42.0		43.2		60.1		50.0		33.2		35.9	289.8	41.40
304			64.2		50.6		30.8		43.1		42.0		34.9		265.6	44.27
205	Straw + $NaNO_3$‡	20.2		43.0		39.6		58.8		45.0		36.9		39.0	282.5	40.36
305			63.2		52.2		31.7		46.4		38.9		34.7		267.1	44.52
206	$NaNO_3$	25.1		46.3		38.5		62.7		42.4		36.5		36.3	287.8	41.11
306			56.5		51.3		30.7		42.4		36.8		33.7		251.4	42.35
207	Straw + $(NH_4)_2SO_4$‡	16.2		46.3		41.0		61.7		46.7		42.9		32.7	287.5	41.07
307			59.6		52.4		32.4		46.0		43.0		34.9		268.3	44.72
208	Manure	21.8		41.0		41.4		62.2		46.0		35.2		35.0	282.6	40.37
308			51.0		35.8		27.9		46.1		33.7		35.2		229.7	38.28

* Fertilizer applied in the spring.

† Fertilizer applied in the fall.

‡ Fertilizer applied at time of plowing for summer fallow.

drop in yields indicated for the plots in the 200 and 300 series was not caused by declining soil productivity but rather by frequent lodging of the crops.

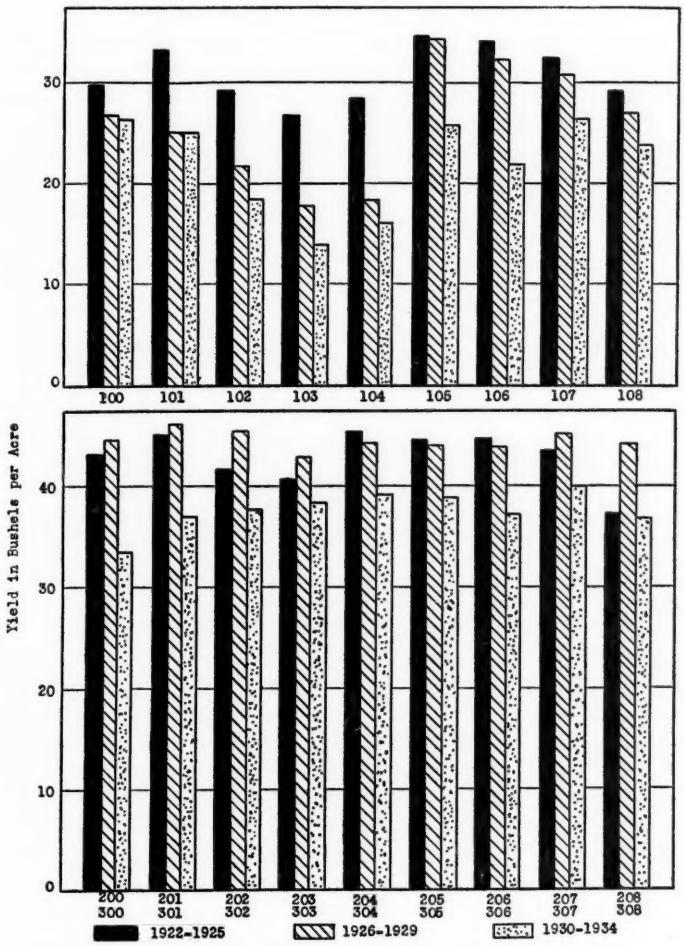

FIG. 1. TREND IN AVERAGE YIELDS OF WHEAT IN SUCCESSIVE PERIODS

No signs of declining productivity, in so far as the appearance of the crop is concerned, were demonstrated.

If the nitrogen content of the grain is a criterion of quality for wheat, the different treatments involving various organic residues and nitrogen fertilizers which supply a substantial quantity of nitrogen to the soil might be

* Fertilizer applied in the spring.
† Fertilizer applied in the fall.
‡ Fertilizer applied at time of plowing for summer fallow.

expected to affect the quality of the wheat produced. The nitrogen content of each crop produced on individual plots was determined, and the averages for each plot for the 13-year period are shown graphically in figure 2. Without exception, the treatments which contributed a substantial quantity of nitrogen to the soil in the form of organic residues or nitrogen fertilizers applied singly or in combinations resulted in an increased average nitrogen content of the grain. The increases resulting from this added nitrogen were greater, however, for the wheat produced on the 100 series which was cropped annually than for the wheat produced on the other two series where cropping was alternated with summer-fallow and where available nitrogen had a chance to accumulate during the summer-fallow year.

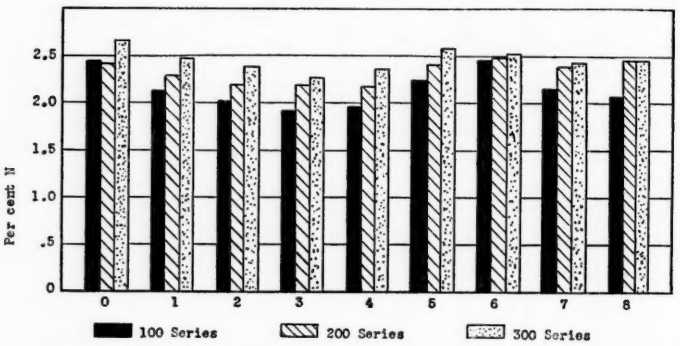

FIG. 2. AVERAGE PERCENTAGES OF NITROGEN IN THE GRAIN PRODUCED

Effect of different treatments on soil humus

Since soil nitrogen exists principally in the form of organic matter and the carbon-nitrogen ratio in the humus of Palouse silt loam under its environmental climatic conditions is fairly constant, the amount of nitrogen nitrified in connection with humus decomposition must be associated with a proportional loss of carbon. When soluble or nitrified nitrogen is removed by crops or lost by leaching, it can no longer function in the formation of new humus. According to Sievers and Holtz (4) the carbon-nitrogen ratio of the organic matter in Palouse silt loam becomes comparatively stable when decomposition has resulted in the reduction of this ratio to 10 or less, a value which approaches the carbon-nitrogen ratio of the soil microflora. Thus, in Palouse silt loam which has been under the alternate summer-fallow and wheat system of cropping for a period of years and, therefore, has received only a relatively small amount of crop residues, approximately nine parts of carbon should be lost for every part of nitrogen that is nitrified or assimilated by crops, and the carbon-nitrogen ratio of the remaining humus should become narrower and eventually approach the theoretical ultimate ratio. Additions of any crop residue, includ-

ing roots, stubble, or other organic substances, can be expected to contribute to the humus supply in this soil only in so far as the nitrogen it contains together with the soluble soil nitrogen which has not been lost by leaching or assimilated by growing crops will combine with carbon in the same rate in which it occurs in humus. The remaining carbon of the added organic residues will be lost rapidly as CO_2. Organic residues with a high nitrogen content or highly carbonaceous residues supplemented with nitrogen fertilizers should contribute, therefore, more soil humus than do organic residues with a low nitrogen content such as straw, for example. In view of the foregoing consideration the data on carbon and nitrogen which have resulted from the different cropping practices and treatments employed for 13 years in this investigation should be highly significant. The carbon-nitrogen ratios will be discussed first and the behavior of the nitrogen and carbon in the soil subsequently.

Carbon-nitrogen ratios

If repeated cultivation with a minimum return of undecomposed crop residues to the soil results in a stimulation of the microbial activity with a consequent accelerated loss of humus and a reduction of the carbon-nitrogen ratio of the remaining soil humus, the carbon-nitrogen ratio of the humus in the check plots under the alternate summer-fallow and wheat system of cropping should have a tendency to become narrower as time progresses. On the other hand, if humus with a carbon-nitrogen ratio considerably greater than the values of 9 to 13 suggested by Sievers and Holtz (3) can be produced in Palouse silt loam under its natural environment, the carbon-nitrogen ratio of the humus in the plots receiving various organic residues, and particularly in those plots which received these residues annually, should become wider rather rapidly as time progresses. The widening of this ratio would depend principally upon the amount of available carbon supplied by the organic residues independent of their nitrogen content. In other words, the addition of straw should be just as effective in the production of humus as the addition of farm manure or alfalfa hay. The carbon-nitrogen ratios of the humus in the different plots in 1921 and in 1934, 13 years later, show (table 3) a remarkable uniformity regardless of the system of cropping practised or the nature of the soil treatments employed, except that of the humus in plot 307, which is slightly narrower in 1934 than in 1921. The discrepancy in this case is so small, however, that it may be within the experimental error.

The effect of the alternate cropping and summer-fallowing on the check plots in the 200 and 300 series, which received no other organic residues than the roots and stubble of the wheat produced on them, was insufficient to reduce the carbon-nitrogen ratio of the soil humus. Evidently, the quantity of roots and stubble returned to the soil in alternate years was sufficient to overcome the aforementioned tendency to reduction of the carbon-nitrogen ratio of humus upon more complete decomposition. Neither were any of the additions

of the various organic residues to the soil effective in widening the carbon-nitrogen ratios of the humus significantly even in the plots of the 100 series where the residues were applied annually at the rate of 2,700 pounds per acre. These data offer strong evidence in support of the foregoing theoretical considerations since in Palouse silt loam in its natural environment humus appeared to be produced only in the ratio of approximately 11 to 12 parts of carbon to 1 part of nitrogen. In the process of decomposition of organic residues added to this soil, therefore, all the carbon supplied in excess of this ratio must have been dissipated rapidly by CO_2 evolution.

Behavior of nitrogen in the soil

The two chief sources of loss of soil nitrogen are leaching and crop removal. The data on the behavior of nitrogen in the surface soil of the various plots during the experimental period are recorded in table 4, in which are shown the

TABLE 3

Carbon-nitrogen ratios of the humus in the different plots in 1921 and 1934

TREATMENT	PLOT NUMBER	C/N		PLOT NUMBER	C/N		PLOT NUMBER	C/N	
		1921	1934		1921	1934		1921	1934
Straw + $NaNO_3$	100*		11.7	200*		11.9	300*		12.0
Alfalfa	101	11.2	11.9	201	11.3	12.0	301*		12.0
Alfalfa + straw	102	11.7	12.1	202	11.2	11.6	302	11.4	12.2
Straw	103	11.4	11.5	203	10.4	11.9	303	11.2	11.8
Check	104	11.7	11.7	204	10.9	11.9	304	10.8	11.6
Straw + $NaNO_3$	105	11.4	11.6	205	11.3	11.4	305	11.1	11.5
$NaNO_3$	106	11.8	12.1	206	10.7	11.9	306	11.2	11.3
Straw + $(NH_4)_2SO_4$	107	11.4	12.2	207	11.2	11.7	307	11.7	11.5
Manure	108	11.5	12.0	208	10.7	11.4	308	10.5	11.6

* Soil samples for 1921 lost.

amounts of nitrogen removed by cropping and those added by means of organic materials and fertilizers. The actual gain or loss of nitrogen from the plowed layer after the amounts removed by the crops and the amounts added by organic residues and fertilizers have been accounted for appear in the last column. It is realized that some of the nitrogen removed by the crops was obtained from the subsoil below the plowed layer, but the error thus introduced is common to all the plots and, therefore, should not interfere seriously with comparative values. All the values in table 4 and also those in tables 5 and 6 on the behavior of carbon and nitrogen, to be discussed later, were calculated on the basis of 2,000,000 pounds of dry soil per acre, the assumption being that this weight represents the plowed layer which was 6 to 7 inches deep and sampled to a depth of 6 inches.

The data in table 4 show that in all the plots in the 100 series, except plot 103, more nitrogen was returned to the soil by means of the various treatments

TABLE 4

Nitrogen content of soils and effect of treatments on nitrogen balance, based on 2,000,000 pounds of dry soil per acre

PLOT NUMBER	TREATMENT	NITROGEN, IN POUNDS PER ACRE						
		Removed by grain and straw	Added as residues and fertilizers*	Gain or loss	In the soil		Gain or loss	Balance, gain or loss†
					1921	1934		
100‡	Straw + $NaNO_3$‖	608.1	884	275.9		3,204		
101	Alfalfa	508.9	702	193.1	3,270	3,352	82	−111.1
102	Alfalfa + straw	317.6	403	85.4	3,204	3,232	28	−57.4
103	Straw	334.8	104	−230.8	3,386	3,334	−52	178.8
104	Check	369.8	000	−369.8	3,260	3,088	−172	197.8
105	Straw + $NaNO_3$§	666.6	884	217.4	3,318	3,432	114	−103.4
106	$NaNO_3$	644.7	780	135.3	3,404	3,272	−132	−267.3
107	Straw + $(NH_4)_2SO_4$§	608.5	884	275.5	3,420	3,518	98	−177.5
108	Manure	526.9	780	253.1	3,432	4,022	590	336.9
200‡	Straw + $NaNO_3$‖	480.1	476	−4.1		2,848		
201	Alfalfa	469.7	378	−91.7	3,420	2,900	−520	−428.3
202	Alfalfa + straw	449.4	217	−232.4	3,460	2,992	−468	−235.6
203	Straw	419.8	56	−363.8	3,476	3,002	−474	−110.2
204	Check	465.3	000	−465.3	3,420	2,928	−492	−26.7
205	Straw + $NaNO_3$**	514.1	476	−38.1	3,404	3,118	−286	−247.9
206	$NaNO_3$	524.6	420	−104.6	3,632	3,002	−630	−525.4
207	Straw + $(NH_4)_2SO_4$**	507.5	476	−31.5	3,420	3,118	−302	−270.5
208	Manure	508.6	420	−88.6	3,632	3,290	−342	−253.4
300‡	Straw + $NaNO_3$‖	480.4	408	−72.4		3,036		
301‡	Alfalfa	486.0	324	−162.0		3,068		
302	Alfalfa + straw	435.7	186	−249.7	3,444	3,002	−442	−192.3
303	Straw	423.1	48	−375.1	3,404	3,060	−344	31.1
304	Check	452.3	00	−452.3	3,546	3,032	−514	−61.7
305	Straw + $NaNO_3$**	494.2	408	−86.2	3,404	3,088	−316	−229.8
306	$NaNO_3$	460.5	360	−100.5	3,340	2,992	−348	−247.5
307	Straw + $(NH_4)_2SO_4$**	468.0	408	−60.0	3,158	3,078	−80	−20.0
308	Manure	413.0	360	−53.0	3,546	3,306	−240	−187.0

* The figures for nitrogen in the straw were computed from averages of a large number of determinations on straw produced in different years, whereas those for nitrogen in the alfalfa hay and in the manure were computed from average nitrogen contents of these materials used in two different years.

† N balance figures show gain or loss of nitrogen in the soil after accounting for the nitrogen removed by the crops and the nitrogen added by organic residues and fertilizers.

‡ Soil samples for 1921 lost.

‖ Fertilizer applied in the spring.

§ Fertilizer applied in the fall.

** Fertilizer applied at the time of plowing for summer-fallow.

than was removed by the crops, whereas in all the plots of the 200 and 300 series more nitrogen was removed by the crops produced than was added by means of the organic materials and fertilizers. It is indicated also that not all the nitrogen lost from the plowed layer was confined to that removed by the crops; some of it disappeared by other means. All plots receiving soluble nitrogen in the fall lost greater quantities of this element than was removed by the crops. Evidently some of it was leached to lower levels during the dormant season when rainfall is most abundant. In general the loss by leaching was considerably greater in the plots of the 200 and 300 series, where the accumulation of nitrate-nitrogen was possible during the summer-fallow period, than that in the plots of the 100 series, where on account of lack of moisture during the growing season microbial activity and decomposition of organic matter were greatly curtailed. The loss of nitrogen in excess of the amounts removed by crops was not entirely confined to the plots receiving soluble nitrogen in the form of fertilizers. Those treated with alfalfa hay and manure also suffered substantial losses of this character, particularly in the 200 and 300 series under the alternate summer-fallow and wheat system of cropping. Even the treatments with a mixture of equal parts of alfalfa hay and straw resulted in a substantial loss under this system of cropping but not under annual cropping in the 100 series. It appears that the rate of decomposition of the manure, of the alfalfa hay, and of the mixture of alfalfa hay and straw in the soil of the plots of the 200 and 300 series, and of the alfalfa hay in the soil of the plots in the 100 series, proceeded rapidly enough to result in the accumulation of some nitrate-nitrogen before or during the early part of the rainy season, so that downward movement of soluble nitrogen by percolation was possible.

The behavior of the nitrogen in the surface soil of the check plots and the straw-treated plots is noteworthy. An actual increase in total nitrogen was realized in these plots in the 100 series but not in the 200 and 300 series where the magnitude of loss or gain in nitrogen is within the experimental error. The difference in the behavior of the nitrogen under the two different cropping systems may be the result of the particular function of the nonsymbiotic nitrogen-fixing bacteria. The results published elsewhere (5, 6) of laboratory studies relating to the activity of *Azotobacter* in the soil of some of these plots show that these organisms were most active in the early spring months and also in the fall, and that in the presence of an abundance of readily available, highly carbonaceous materials they were capable of fixing large amounts of atmospheric nitrogen. Both the check plot and the straw treated plot in the 100 series contained an abundance of readily decomposable, highly carbonaceous organic residues in the form of plant roots and straw during the fall months. Since the supply of available soil nitrogen was limited under the prevailing conditions, and organic matter decomposition proceeds very slowly during the winter months, some of the supply of available carbohydrates probably was carried over for use in the early spring months when soil moisture

was still plentiful and *Azotobacter* most active, thus providing optimum conditions for the fixation of atmospheric nitrogen. This may account in part for the gain in nitrogen in the soil of these plots. The cropping system on the plots in the 200 and 300 series was such that the most active decomposition of organic matter occurred during the summer months when the activity of *Azotobacter* appeared to be low and, hence, nitrogen fixation by these organism might be expected to be proportionately low, with the result that no appreciable gain in nitrogen was realized.

Behavior of carbon and humus in the soil

The data on changes in the carbon content of the soil in the various plots disregarding the carbon supplied by plant roots and stubble are presented in table 5. If the liberation of nitrogen by the decomposition of humus is associated with a proportional loss of carbon, which in this soil should be approximately 11 to 12 parts of carbon to 1 part of nitrogen, the loss of soil humus should be greater in the plots of the 200 and 300 series under alternate cropping and summer-fallow than in the plots of the 100 series. That this was the case is shown in table 5, which also indicates that the addition of organic residues to the plots in the 100 series counteracted this loss to the extent that some increase in the carbon content of the soil was realized except in plot 103 where the application of straw alone had practically no effect in this respect. The data show further that the gain in carbon content was roughly proportional to the quantity of nitrogen supplied by the organic residues or as a supplement to these residues. The addition of straw alone, which supplied as much carbon as the other residues, except manure, but very little nitrogen, failed to cause any increase in the carbon content of the soil, whereas the application of a mixture of alfalfa hay and straw, which supplied a similar amount of carbon and about half as much nitrogen as the treatments with manure, alfalfa hay, and straw supplemented with nitrogen fertilizers, resulted in an increased carbon content of the soil roughly proportional to the amount of nitrogen added. Some deviation from this general trend occurred in the plot treated with straw supplemented with sodium nitrate, but experimental errors incidental to soil sampling and chemical analysis may account for part of the discrepancy. A substantial amount of humus was lost from plot 103, the untreated plot, and very little from plot 106, which received nitrogen fertilizer. The soluble nitrogen added to this plot probably was instrumental in fixing more of the carbon contributed to the soil by plant roots and stubble than could be fixed in the soil of the check plot in which the liberated nitrogen was quickly assimilated by the crops.

Since the plots treated with organic residues in the 100 series received twice as much material as the corresponding plots in the 200 and 300 series during the experimental period, the effect of these treatments should be more pronounced in the former than in the latter. Under annual cropping with winter wheat the moisture content in the plowed layer of the plots in the 100 series

TABLE 5

Carbon content of soils and effect of treatments on the carbon balance, based on 2,000,000 pounds of dry soil per acre

PLOT NUMBER	TREATMENT	CARBON, IN POUNDS PER ACRE				
		In the soil		Gain or loss	Added by residues*	Actual loss†
		1921	1934			
100‡	Straw + $NaNO_3$‖		37,460		12,271	
101	Alfalfa	36,740	39,960	3,220	12,271	9,051
102	Alfalfa + straw	37,500	39,160	1,660	12,271	10,611
103	Straw	38,780	38,220	−560	12,271	12,831
104	Check	38,040	36,000	−2,040		2,040
105	Straw + $NaNO_3$‖	37,960	39,860	1,900	12,271	10,371
106	$NaNO_3$	40,080	39,620	−460		460
107	Straw + $(NH_4)_2SO_4$§	38,880	43,020	4,140	12,271	8,131
108	Manure	39,640	48,480	8,840	17,316	8,476
200‡	Straw + $NaNO_3$‖		33,860		6,607	
201	Alfalfa	38,540	34,820	−3,720	6,607	10,327
202	Alfalfa + straw	38,900	34,640	−4,260	6.607	10,867
203	Straw	38,720	35,600	−3,120	6,607	9,727
204	Check	37,340	34,820	−2,520		2,520
205	Straw + $NaNO_3$**	38,360	35,660	−2,700	6,607	9,307
206	$NaNO_3$	38,900	35,600	−3,300		3,300
207	Straw + $(NH_4)_2SO_4$**	38,220	36,560	−1,660	6,607	8,267
208	Manure	38,900	37,680	−1,220	9,224	10,444
300‡	Straw + $NaNO_3$‖		36,920		5,664	
301‡	Alfalfa		36,960		5,664	
302	Alfalfa + straw	39,260	36,500	−2,760	5,664	8,424
303	Straw	38,160	36,160	−2,000	5,664	7,664
304	Check	38,340	35,060	−3,280		3,280
305	Straw + $NaNO_3$**	37,880	35,660	−2,220	5,664	7,884
306	$NaNO_3$	37,460	33,860	−3,600		3,600
307	Straw + $(NH_4)_2SO_4$**	36,860	35,540	−1,320	5,664	6,984
308	Manure	37,140	38,320	1,180	7,992	6,812

* Calculated from an average of several carbon contents of the straw, alfalfa hay, and manure used in two different years.

† The figures for actual loss represent the difference between the amounts of carbon in the soil in 1934, and the sum of the carbon in the soil in 1921 and the amounts supplied by means of the organic residues.

‡ Soil samples for 1921 lost.

‖ Fertilizer applied in the spring.

§ Fertilizer applied in the fall.

** Fertilizer applied at the time of plowing for summer-fallow.

was near or below the wilting point most of the time from May 15 until the fall rains came late in September or, as more often happened, in October. Consequently, the microbial activity was greatly reduced, with the result that organic matter decomposition and the liberation of nitrogen were re-

stricted largely to the late fall and early spring months. An entirely different condition prevailed in the plowed layer of the plots in the 200 and 300 series. The type of summer-fallow practised in alternate years when the organic residues were applied resulted in the maintenance of optimum moisture conditions at a time when temperature was ideal for maximum microbial activity; hence, a more intensive breakdown or organic matter and, consequently, a greater loss of carbon from the soil took place. These assumptions are in agreement with the data in table 5, which indicate that in general the additions of organic residues to the plots in the 100 series caused an increase in the carbon content of the soil, whereas in only one plot, plot 308, which received manure, was the addition of organic residues to the plots in the 200 and 300 series effective in this respect. The slight gain in plot 308 probably is attributable to experimental error in soil sampling and analysis. The loss from the other plots treated with organic residues in these two series was distinct and occurred in fairly comparable amounts in plots receiving similar treatments. It was least, however, from the manured plots and relatively low also from the plots treated with straw supplemented with ammonium sulfate. The relatively small losses of carbon resulting from these treatments in the 200 and 300 series correspond with proportionally greater gains in carbon from similarly treated plots of the 100 series. For some reason, yet obscure, more humus seemed to be fixed in the process of decomposition of these products than was fixed in the process of decomposition of the other organic residues.

Although in the 100 series the application of nitrogen fertilizer alone effected a marked reduction in the loss of humus, the same treatment in the 200 and 300 series proved to be ineffective in this respect, probably because the amount of nitrogen liberated during the summer-fallow year caused the breakdown of a greater quantity of soil humus than could be fixed by the transformation of the limited amount of root and stubble material supplied by the previous crop.

Carbon and nitrogen relationships

The actual changes in the humus content of the soil in the various plots as portrayed by the data on carbon contents in table 5 show convincingly that the alternate summer-fallow and wheat system of cropping was much more destructive to humus than was the system of annual cropping with wheat. A thorough consideration of the total amounts of nitrogen as well as of carbon which disappeared from the surface soil should offer an approach to a more accurate quantitative estimation of the behavior of the soil humus and the transformation of the organic residues added to the soil under the two different systems of cropping. The difficulties involved in subjecting the products of the complex processes of nitrogen and carbon transformation in the soil to strict mathematical treatment are obvious and are fully appreciated in this connection. Nevertheless, the data presented thus far offer convincing proof that the carbon-nitrogen ratio of the soil humus did not change appreciably

during the experimental period regardless of soil treatment, so that the new humus produced from organic residues added in various forms as well as that destroyed by decomposition probably was of similar composition. Thus, if the amounts of both carbon and nitrogen lost from the soil are known it should be possible by means of calculations to make a rough estimate of how much of the carbon added to the soil in the form of organic residues was transformed to humus and how much was dissipated by CO_2 evolution. The amounts of carbon in the organic residues used for the various soil treatments can be calculated. Those supplied by means of plant roots and stubble, however, are an unknown factor, but the quantities thus supplied probably were relatively uniform for the various plots in any one series. The results of calculations of the total amounts of carbon lost on the basis of the average carbon-nitrogen ratio for the humus of all the plots, taking into consideration also the total carbon added to the various plots by means of organic residues, should be highly significant, therefore, even though not exactly accurate from a quantitative standpoint. These calculations were made, and the results are presented in table 6.

The figures in the third column of table 6 represent the actual loss of nitrogen, and those in the fourth column, the actual loss of carbon from the plowed layer of the various plots. The data in the fifth column indicate how much of the lost carbon was derived from humus with an assumed carbon-nitrogen ratio of 11.8 on the basis of the total loss of nitrogen from the surface soil. The positive values in the last column give an indication of the magnitude of the loss of carbon by CO_2 evolution from organic material which probably was not transformed to humus. The negative values in this column are explainable on the basis that some of the soluble nitrogen added to the soil by means of fertilizers, particularly that added to the plots which received no organic residues, was leached to lower depths or assimilated by the crops before it could be used in the transformation of organic matter to humus, and also that some of the carbon supplied by plant roots and stubble was transformed to humus.

A careful examination of the data in this last column reveals that in each of the three series those plots which received straw alone or a mixture of straw and alfalfa hay suffered most from loss of carbon in excess of the assumed carbon-nitrogen ratio of 11.8, but the loss from straw alone was more pronounced than that from the mixture of straw and alfalfa hay. It is noted also that the loss of carbon in proportion to the amount applied to the soil was greater under continuous cropping than under the alternate summer-fallow and wheat system of cropping. In plot 103, for example, the carbon lost in excess of the carbon-nitrogen ratio of 11.8 was about 89 per cent of the total amount applied to it in the form of straw, whereas the value for plots 203 and 303 respectively was about 60 per cent. The less pronounced loss of carbon from the plots receiving the mixture of straw and alfalfa hay occurred in a similar relationship. The values indicated are about 53 per cent for plot

102 and approximately 42 and 20 per cent for plots 202 and 302 respectively. The losses of carbon in excess of the carbon-nitrogen ratio of 11.8 from the

TABLE 6

Loss of carbon and nitrogen in the ratio of (C/N = 11.8) and loss of carbon above or below this ratio, based on 2,000,000 pounds of dry soil per acre

PLOT NUMBER	TREATMENT	N LOST, IN POUNDS PER ACRE*	CARBON, IN POUNDS PER ACRE		
			Lost†	Lost as (C/N=11.8)‡	Above or below (C/N=11.8)‖
101	Alfalfa	694	9,051	8,189.2	861.8
102	Alfalfa + straw	375	10,611	4,425.0	6,186.0
103	Straw	156	12,831	1,840.8	10,990.2
104	Check	172	2,040	3,029.6	−989.6
105	Straw + $NaNO_3$	770	10,371	9,086.0	1,285.0
106	$NaNO_3$	912	460	10,761.6	−10,301.6
107	Straw + $(NH_4)_2SO_4$	786	8,131	9,274.8	−1,143.8
108	Manure	190	8,476	2,242.0	6,234.0
201	Alfalfa	898	10,327	10,596.4	−269.4
202	Alfalfa + straw	685	10,867	8,083.0	2,784.0
203	Straw	530	9,727	6,254.0	3,473.0
204	Check	492	2,520	5,805.6	−3,285.6
205	Straw + $NaNO_3$	762	9,307	8,991.6	315.4
206	$NaNO_3$	1,050	3,300	12,390.0	−9,090.0
207	Straw + $(NH_4)_2SO_4$	778	8,267	9,180.4	−913.4
208	Manure	762	10,444	8,991.6	1,452.4
302	Alfalfa + straw	628	8,424	7,410.4	1,013.6
303	Straw	392	7,664	4,625.6	3,038.4
304	Check	514	3,280	6,065.2	−2,785.2
305	Straw + $NaNO_3$	724	7,884	8,543.2	−659.2
306	$NaNO_3$	708	3,600	8,354.4	−4,754.4
307	Straw + $(NH_4)_2SO_4$	488	6,984	5,758.4	1,225.6
308	Manure	600	6,812	7,080.0	−268.0

* The figures for nitrogen lost represent the difference between the amounts of nitrogen present in the surface soil in 1934, and the sum of the nitrogen present in 1921 and the amounts supplied by means of crop residues and fertilizers.

† The figures for carbon lost represent the difference between the amounts of carbon present in the surface soil in 1934, and the sum of the carbon present in 1921 and the amounts supplied by means of organic residues.

‡ Carbon lost via humus stage which is assumed to be 11.8 x N lost.

‖ The plus values indicate the amounts of carbon lost in excess of the C/N of 11.8, and the minus values, the amounts of carbon lacking to make the loss equivalent to that of this ratio. The carbon supplied to the soils by means of plant roots and stubble is an unknown factor.

other residues, all of which supplied much greater quantities of nitrogen than did the straw or the mixture of straw and alfalfa hay, either by virtue of their composition or of the fact that supplemental nitrogen was added by means

of fertilizers, appear to be somewhat irregular but, in general, are much smaller than those for the straw or the mixture of straw and alfalfa hay residues. Since the calculations involved in obtaining these values tend to magnify the experimental errors incidental to soil sampling and chemical analysis, the irregularities of these relatively small values and even of the negative values indicated in some cases are not significant.

The significant point brought out by these data is that the influence of available nitrogen in the formation of humus from undecomposed organic residues is convincingly demonstrated by the fact that loss of carbon in excess of the assumed carbon-nitrogen ratio of 11.8 appeared to be in proportion to the amount of nitrogen contained in the residues or supplied with them by nitrogen fertilizers. As pointed out previously, it was greatest in the plots receiving straw alone, indicating that the major part of the carbon in the straw was not transformed to humus but was dissipated by CO_2 evolution. These results corroborate the contention of Sievers and Holtz (3). The loss of carbon from the added straw was less pronounced, however, under the alternate summer-fallow and wheat system of cropping in which some of the nitrogen liberated from the soil humus during the summer-fallow years was available for the formation of new humus from the straw than it was under the system of annual cropping in which crops competed strongly with the soil microflora in the utilization of the liberated nitrogen. When straw was supplemented with a sufficient amount of nitrogen fertilizers to make the available supply of nitrogen similar to that in the alfalfa hay and manure, the amounts of carbon dissipated by CO_2 evolution without transformation to humus were reduced to values comparable with those applying to the plots treated with alfalfa hay and with manure.

The negative values indicated in the last column of table 6 for the check plots and the plots treated with sodium nitrate alone are interesting. It may be observed that the values applying to the plots treated with sodium nitrate are greater than those in connection with the check plots and also that the value for the check plot under continuous cropping with wheat is much less than are the values for the check plots in alternate summer-fallow and wheat. The data in the last column of table 4 show that the surface soil in all these plots, except the check plot in the 100 series, lost more nitrogen than can be accounted for by the amounts removed by crops and those added by means of nitrogen fertilizers. These are the plots for which the greatest negative values are shown in the last column of table 6. Leaching of soluble nitrogen to lower depths during the rainy season is probably responsible for a major part of the nitrogen not accounted for in these plots. The amounts which disappeared in this manner were probably greatest in the plots treated with sodium nitrate, since they contained no undecomposed organic residues, which serve indirectly as a means for a temporary tying up of soluble nitrogen through microbial activity. Inasmuch as the nitrogen lost by leaching could not take part in the formation of humus the apparent negative values indicated in the

last column of table 6 for these plots and also for the check plots in the 200 and 300 series are too high. In actuality they would be reduced probably in direct proportion to the leached nitrogen if the quantities were known, leaving final values comparable with the one indicated for the check plot in the 100 series. The quantities of organic residues added to the soil by means of roots and stubble should, therefore, be more than ample to fix humus in the amounts necessary to use up the carbon indicated by negative values of this magnitude.

DISCUSSION

A general consideration of the data obtained in this investigation, which covered a period of 13 years, reveals useful information applicable in practical agriculture and in sound soil management practices. The results offer convincing evidence that the alternate summer-fallow and wheat system of farming in semiarid areas, although conducive to the liberation of ample quantities of nitrogen and, therefore, to the production of large yields of wheat, is highly destructive to the humus supply of the soil. When proper moisture conditions are maintained in the surface soil by suitable summer-fallow practices, the loss of humus by decomposition is so rapid that it cannot be prevented fully by applications of organic residues in moderate amounts consistent with field practice.

The data pertaining to the effect of various organic residues on yields of wheat show that, whereas under the alternate summer-fallow and wheat system of cropping no appreciable increases in yields resulted from applications of these materials, marked increases in yields were obtained under the system of annual cropping with wheat. Available nitrogen proved to be a limiting factor under the annual system of cropping. The increases in yield were generally proportional to the amount of nitrogen added to the soil regardless of whether it was supplied in the form of organic residues or in the form of nitrogen fertilizers. In spite of the beneficial effect of added nitrogen to the soil, yields gradually declined as time progressed; consequently, annual cropping with wheat even when adequate quantities of nitrogen are supplied to the soil is not likely to be a profitable practice.

The most important data with respect to the maintenance of the humus supply and the fertility of Palouse silt loam in its natural climatic environment are those showing the effect of applications of various organic residues on the humus content of the soil. The results obtained lead to the conclusion that in the process of decomposition of organic residues added to the soil under field conditions the formation of humus occurs in the ratio of approximately 12 parts of carbon to 1 part of nitrogen. Any carbon supplied by organic residues in excess of this ratio is rapidly dissipated by CO_2 evolution without being transformed to humus. Thirteen annual applications of straw used at a rate approximately equivalent to that produced by a wheat crop yielding about 35 bushels per acre were ineffective in increasing the humus content of the soil.

Although the practice of returning straw to this soil and to other soils under similar climatic condition may have some beneficial mechanical effect on the soil and be instrumental in reducing the rate of loss of humus under the prevailing cropping practices, it cannot be expected to add materially to the humus supply of the soil unless the straw is supplemented with sufficient nitrogen fertilizer or other nitrogenous materials to make the total amount of available nitrogen approximately equivalent to that of alfalfa hay. Since the nitrogen made available in the soil for crop growth is derived principally from the decomposition of humus, the amount of nitrogen returned to the soil by means of crop residues or as a supplement to these crop residues must be sufficient to produce as much humus as is being destroyed, in order to prevent the gradual depletion of soil humus by means of cropping and cultivation.

SUMMARY

The results of laboratory studies on CO_2 evolution and nitrate-nitrogen accumulation indicated a possible relationship between available nitrogen and the formation of humus in the soil.

Applications of various organic residues under field conditions or of nitrogen fertilizers alone or as a supplement to the organic residues did not result in appreciable increases in yield under alternate cropping and summer-fallow but, on an average, caused substantial increases in yields of wheat under annual cropping, the increases being roughly proportional to the amounts of nitrogen applied to the soil. Yields under annual cropping gradually declined, regardless of soil treatments.

The addition of substantial quantities of nitrogen to the soil in various forms resulted in an increased average nitrogen content of the grain. Not all the nitrogen disappearing from the plowed layer could be accounted for by the amounts assimilated by crops. Probably some of it was lost by leaching to lower depths, and it appears that this loss was considerably greater under alternate cropping and summer-fallow than under annual cropping.

The carbon-nitrogen ratio of the soil humus in the various plots appeared to be slightly greater after 13 years of soil treatment than before such treatment but remained remarkably uniform in all plots regardless of the system of cropping practised or of the nature and amount of crop residues and nitrogen fertilizers applied to the soil.

Annual applications of organic residues, except the application of straw alone, were effective in increasing the humus content of the soil under annual cropping with wheat. The gain in humus was roughly proportional to the amount of nitrogen supplied to the soil by means of organic residues or as a supplement to these residues.

A marked loss of soil humus occurred under alternate cropping and summer-fallow in spite of additions of organic residues. The loss of humus by microbial activity was so great during the summer-fallow year that it could not be overcome fully by applications of organic residues in amounts consistent with field practice.

Convincing evidence was produced to show that the decomposition of organic residues in Palouse silt loam under its natural climatic environment results in the formation of humus with a carbon-nitrogen ratio of approximately 12. Any carbon added in excess of this ratio by means of organic residues was rapidly dissipated by CO_2 evolution.

The practice of returning straw to this soil, although having a beneficial physical effect on the soil and causing a decline in the rate of loss of soil humus under the prevailing cropping practices, cannot be expected to result in the production of substantial quantities of new humus unless the straw is supplemented with some form of available nitrogen in sufficient amounts to make the total supply approximately equivalent to that in alfalfa hay.

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STUDIES IN SOIL HUMUS: II. POTENTIOMETRIC STUDY OF THE FORMATION OF HUMIC ACID AND HUMATES

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The alkali-soluble humus in soils constitutes a group of substances that are distinct from the rest of the organic matter. The most important common property of this group is that its members exhibit acidic properties and their alkali salts are soluble in water. It does not seem to be sufficiently recognized that when we speak of the solubility of humus in alkalies we refer to the formation of the alkali salts of humic acid. This fact has been ignored so much that inorganic materials in soil humus are generally regarded as impurities. It appeared, therefore, that a knowledge of the formation of humic acid and various humates with particular reference to the reaction of the medium would lead to a better understanding of the role of humus in soils.

HUMATES OF ALKALI METALS

Alkali humates are soluble in water and may be the basis of formation of other humates. The best method of studying the formation of alkali humates is to follow the titration curves of humic acid with alkali hydroxides. Such titration curves are shown in figure 1.

In view of the fact that the neutralization of a weak acid with a strong alkali takes place when the pH value is raised by 4 pH units[1], the neutralization of humic acid takes place at pH 7.5. It will be noticed that a faint but distinct point of inflection occurs in the neighborhood of this pH value in all curves in figure 1. Thus to prepare humates of alkali metals it is only necessary to neutralize the humic acid to pH 7.5. Any alkali added in excess of that amount will be partly hydrolyzed, and the humate formed will exhibit increasing alkalinity according to the amount of alkali added for neutralization. It must be noted that the titration curves of weak acids are not expected to be identical when different bases are used for neutralization. The equivalent point at 4 pH units higher than the initial pH value is only true in the case of strong alkalies. It is for this reason that the equivalent point could be most satisfactorily determined by titration with NaOH. Other alkalies may possibly show slight variations due to differences in the dissociation constants of the salts produced.

[1] This subject will be treated by the author in another paper.

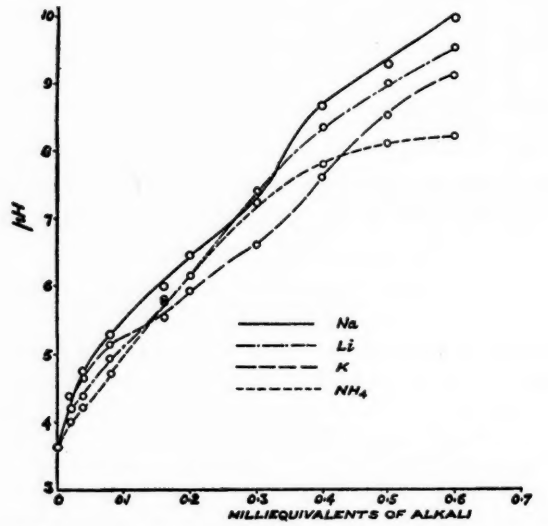

FIG. 1. TITRATION CURVES OF HUMIC ACID WITH ALKALI HYDROXIDES
Humic acid 0.1 gram

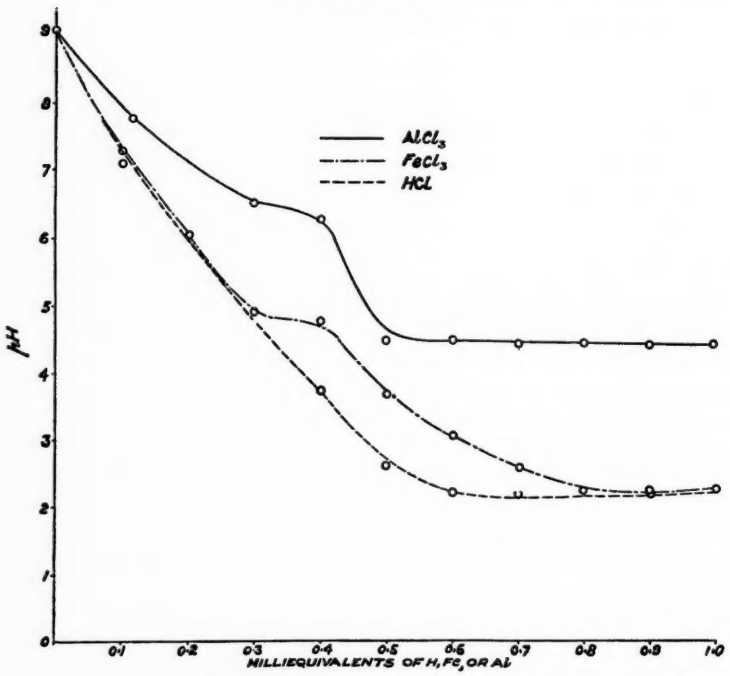

FIG. 2. TITRATION CURVES OF SODIUM HUMATE WITH HCl, $FeCl_3$, AND $AlCl_3$
Sodium humate 0.1 gram

HUMIC ACID

Humic acid is formed by the action of dilute acid on alkali humates. The course of its formation from sodium humate by HCl is shown in figure 2 (titration curve) and figure 3 (precipitation curve).

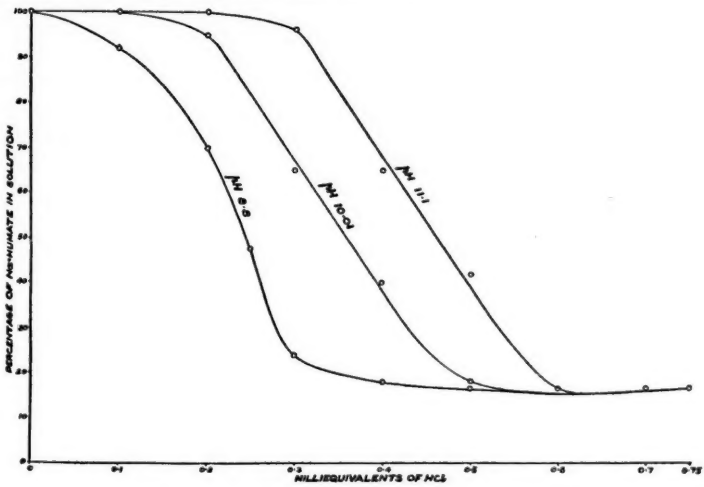

FIG. 3. FORMATION OF HUMIC ACID FROM SODIUM HUMATE
Sodium humate 0.1 gram

TABLE 1

Precipitation of humic acid from Na-humate by HCl

pH of Na-humate.....	8.80		10.01		11.10	
0.1 *N* HCl	pH	Na-humate in solution	pH	Na-humate in solution	pH	Na-humate in solution
cc.		*per cent*		*per cent*		*per cent*
1	5.23	57.5	5.76	100.0	8.13	100.0
2	2.90	22.5	2.94	100.0	5.16	100.0
3	2.07	15.0	2.22	60.2	2.83	100.0
4	1.92	15.0	2.07	38.5	2.37	62.5
5	1.88	15.0	1.84	15.0	1.97	45.0
6	1.75	15.0	1.75	15.0	1.82	15.0
7	1.68	15.0	1.68	15.0	1.75	15.0
8	1.60	15.0	1.60	15.0	1.63	15.0
9	1.55	15.0	1.55	15.0	1.57	15.0

It is seen that the complete precipitation of humic acid takes place when an amount of acid equivalent to the Na in the humate has been added. Thus if we take sodium humate of different pH values, the titration curves are the same, but the precipitation curves are shifted to the right or left so that more

or less acid is required for the precipitation. The precipitation of humic acid with HCl is completed at approximately pH 1.8 (table 1). The beginning of the precipitation, however, depends on the pH of the Na-humate, occurring at a higher pH value for Na-humate of a lower pH and *vice versa*. It is also seen that humic acid is partly soluble even at a pH value as low as 1.5.

HUMATES OF ALKALINE EARTH METALS

Calcium, magnesium, and barium humates can be prepared by the direct neutralization of humic acid with the corresponding hydroxides or by the addition of soluble alkaline earth salts to sodium humate. The former reaction can be followed accurately from the titration curves of humic acid with

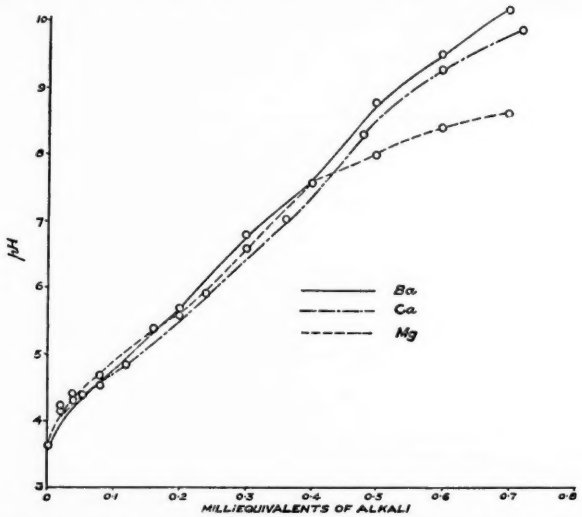

FIG. 4. TITRATION CURVES OF HUMIC ACID WITH ALKALINE EARTH HYDROXIDES
Humic acid 0.1 gram

alkaline earth hydroxides. Such curves are given in figure 4. It is a remarkable fact that insoluble humic acid can react with alkaline earth hydroxides and give a perfect titration curve, in spite of the fact that the products of the reaction are also insoluble.

The precipitation of alkaline earth humates with the corresponding chlorides was also followed potentiometrically. Increasing amounts of $CaCl_2$, $MgCl_2$, and $BaCl_2$ were added to sodium humate solution and shaken for 24 hours, though 2 hours' shaking is sufficient. The pH value of the mixture was determined by the glass electrode, after which the mixture was filtered. An aliquot of the filtrate was taken, and humus was determined by the alkaline permanganate method.[2] The results are plotted in figures 5 and 6, the former

[2] Puri, A. N., and Sarup, A. 1937. Studies in soil humus: I. Estimation of humus by oxidation with alkaline permanganate. *Soil Sci.* 44: 323–327.

giving the pH changes during precipitation and the latter indicating the amount of humus precipitated as humate and that remaining in solution. It

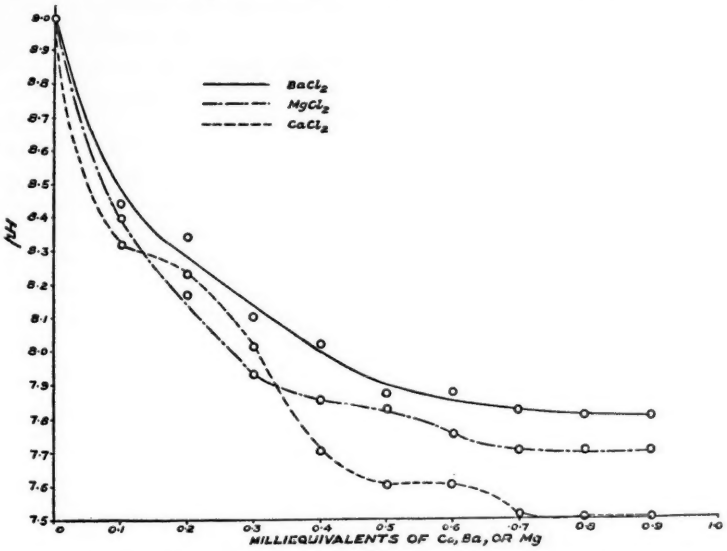

FIG. 5. TITRATION CURVES OF SODIUM HUMATE WITH $CaCl_2$, $BaCl_2$, AND $MgCl_2$
Sodium humate 0.1 gram

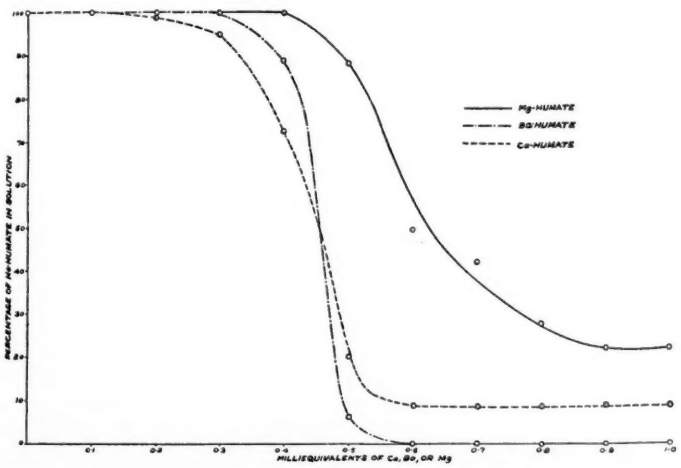

FIG. 6. FORMATION OF Ca-, Ba-, AND Mg-HUMATES FROM SODIUM HUMATE
Sodium humate 0.1 gram

is seen that Ba-humate is the least soluble and that Mg-humate is appreciably soluble.

A careful examination of figures 5 and 6 reveals the fact that a break in the titration curve corresponds to the point at which precipitation of the humate begins. The difference is not very great perhaps, but the abrupt change indicates that the humates of the alkaline earth metals are precipitated in the colloidal state when the pH is high, followed by their subsequent precipitation when the pH value is reduced below a certain minimum. The gradual lowering of the pH value during the addition of alkaline earth salts is partly due to the salt effect and partly to the gradual diminution in the concentration of sodium humate. The non-precipitation of humates at higher pH values may also be due to the peptizing action of the sodium humate. This is confirmed by the fact that the solubility of alkaline earth humates in water is greatly enhanced by the addition of sodium humate. This increased solubility, however, depends on the pH value of the sodium humate: the larger the pH value, the greater the solubility. This will be clear from table 2, which gives the solubility of alkaline earth humates in sodium humate of varying pH values. One-tenth gram of the humate was shaken with 20 cc. of water containing 0.1

TABLE 2

Peptization of alkaline earth humates by Na-humate

pH of Na-humate	Percentage peptization		
	Ca	Ba	Mg
8.19	36.08	49.05	55.8
9.71	57.6	50.5	66.6
10.62	90.0	62.0	92.5
Water	24.8	11.6	52.5

gram of sodium humate. The results are expressed in percentage of the alkaline earth humate dissolved on shaking, the amount brought into solution being determined by titration with alkaline permanganate. It is seen that the peptization is highest with Mg-humate and lowest with Ba-humate. This is exactly what would be expected from the precipitation curves given in figure 6. It must be pointed out that the solubility of alkaline earth humates in water is greatly reduced by the addition of the corresponding salts. This will be clear from a comparison of figure 6 and table 2.

IRON AND ALUMINUM HUMATES

Humates of Fe and Al can be prepared by the addition of $FeCl_3$ and $AlCl_3$ to a solution of sodium humate. The titration curves of sodium humate with $FeCl_3$ and $AlCl_3$ are given in figure 2 along with that for HCl, to which they are similar. In the Fe and Al curves there is an indication of a point of inflection near the equivalent point. One cannot, however, be sure whether the addition of $FeCl_3$ or $AlCl_3$ results in a chemical compound or merely in the simultaneous precipitation of humic acid and iron or aluminum hydroxide. Such a co-

precipitation would result in a solid solution of the two compounds, and if equivalent concentrations had been employed the resulting precipitate would contain iron and aluminum hydroxide and humic acid in stoichiometric proportion; but the stoichiometric proportion would be no proof of chemical combination in this case. If alkali in sodium humate is in excess, the stoichiometric proportion of the precipitate will not hold. As weak alkalies, like iron and aluminum hydroxides, can have but a loose combination with a weak acid like humic acid, the existence of stoichiometric relationship or its absence would be no proof for or against their chemical combination. For instance, in the formation of humic acid by the addition of HCl to sodium humate (fig. 3), the precipitation, as we have seen, does not commence until a considerable

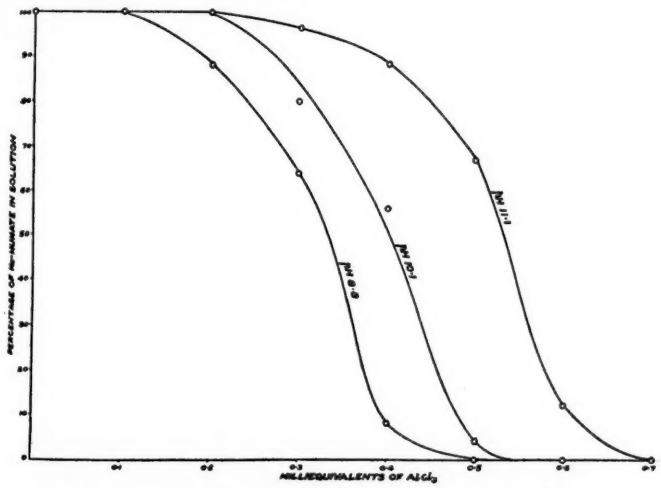

FIG. 7. FORMATION OF ALUMINUM HUMATE FROM SODIUM HUMATE
Sodium humate 0.1 gram

amount of HCl has been added. In other words, we can have sodium humates entirely in solution at different pH values. These humates, if neutralized with $AlCl_3$ or $FeCl_3$, will require different amounts of the latter for complete precipitation of the humate. The precipitated Al- or Fe-humates, however, will contain different proportions of Al or Fe, depending on the pH value of the humate. This is indicated in a striking manner in figures 7 and 8, which show the precipitation of Al- and Fe-humates from sodium humate by the gradual addition of $AlCl_3$ and $FeCl_3$ respectively. The precipitation of Al- and Fe-humate is complete in every case when an amount of $AlCl_3$ or $FeCl_3$ equivalent to the Na in the sodium humate has been added. The resulting precipitates of Al- or Fe-humates, therefore, contain different amounts of Al or Fe. It is an important point that must be borne in mind by all those seeking

stoichiometric proportions in such compounds. The neutralization of acids is a continuous function of the hydrogen-ion concentration. What is true of soluble acids is also true of insoluble acids. Insoluble humic acid can be neutralized with insoluble $CaCO_3$, with the production of insoluble Ca-humate.

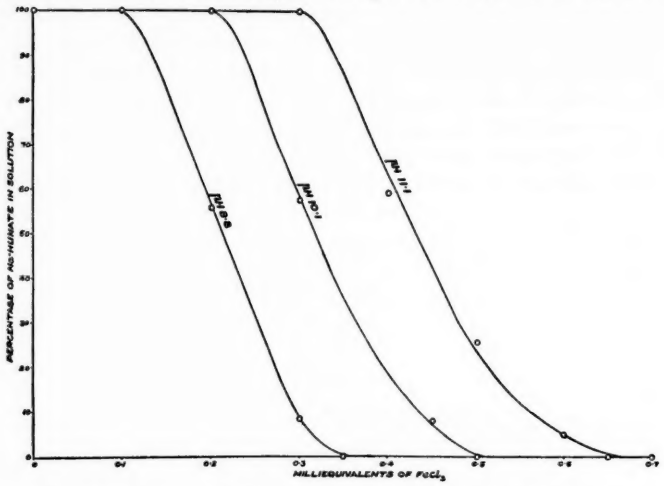

FIG. 8. FORMATION OF FERRIC HUMATE FROM SODIUM HUMATE
Sodium humate 0.1 gram

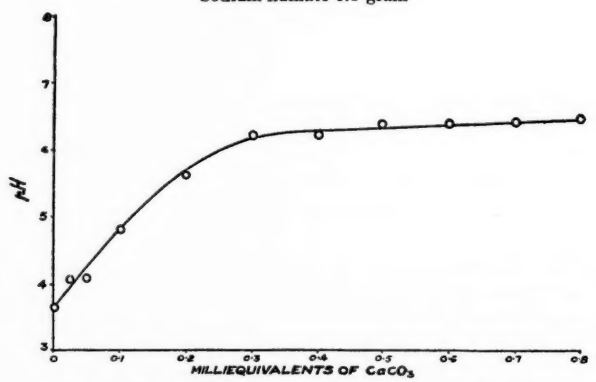

FIG. 9. TITRATION CURVE OF HUMIC ACID WITH $CaCO_3$
Humic acid 0.1 gram

This neutralization follows a titration curve (fig. 9), but the resulting Ca-humate at every step will contain varying amounts of Ca, for in reality it is a perfectly homogeneous mixture of humic acid and Ca-humate and in fact is a solid buffer solution. These perfectly normal compounds are sometimes wrongly designated as adsorption complexes, because of the lack of stoichio-

TABLE 3

Precipitation of Fe-humate from Na-humate by $FeCl_3$

pH of Na-humate.....	8.80		10.01		11.10	
0.1 *N* $FeCl_3$	pH	Na-humate in solution	pH	Na-humate in solution	pH	Na-humate in solution
cc.		*per cent*		*per cent*		*per cent*
1	6.5	100.0	7.41	100.0	8.94	100.0
2	6.30	56.0	6.62	100.0	8.48	100.0
3	6.10	8.0	6.0	57.6	7.17	100.0
4	3.5	0	5.08	20.0	6.15	64.0
5	3.3	0	4.22	8.0	5.66	25.6
6	3.1	0	3.57	0	4.65	5.0
7	2.9	0	2.87	0	3.49	0
8	2.7	0	2.68	0	3.20	0
9	2.5	0	2.58	0	3.0	0

TABLE 4

Precipitation of Al-humate from Na-humate by $AlCl_3$

pH of Na-humate.....	8.8		10.01		11.10	
0.1 *N* $AlCl_3$	pH	Na-humate in solution	pH	Na-humate in solution	pH	Na-humate in solution
cc.		*per cent*		*per cent*		*per cent*
1	6.41	100.0	7.36	100.0	8.70	100.0
2	6.11	88.0	7.05	100.0	8.24	100.0
3	4.71	64.0	6.22	80.0	7.75	96.5
4	3.58	8.0	5.43	51.2	7.23	88.8
5	3.48	0	3.82	4.0	6.46	67.2
6	3.34	0	3.73	0	5.51	12.0
7	3.32	0	3.60	0	4.0	0
8	3.30	0	3.54	0	3.79	0
9	3.25	0	3.50	0	3.70	0

TABLE 5

Solubility of humic acid and of humates in water and in alcohol

NATURE OF HUMUS	HUMUS DISSOLVED IN	
	Water	Alcohol
	per cent	*per cent*
Humic acid	11.6	100.0
Na-humate	100.0	3.92
K-humate	100.0	4.22
Li-humate	100.0	22.76
NH_4-humate	100.0	5.2
Mg-humate	45.5	6.66
Ca-humate	21.06	0.0
Ba-humate	9.72	0.0
Fe-humate	0.0	0.0
Al-humate	0.0	0.0

metric proportions. The results of precipitation of Fe- and Al-humates with respect to pH values are given in tables 3 and 4. A comparison of these results with those given in table 1 shows that precipitation with $FeCl_3$ or $AlCl_3$ takes place at a higher pH value than does precipitation with HCl. It is also seen that Fe- and Al-humates are completely insoluble at a pH value of approximately 3.5. The importance of pH values in all such studies is therefore obvious.

SOLUBILITY OF HUMIC ACID AND HUMATES IN WATER AND ALCOHOL

It is not the object of this paper to discuss the properties of the various humates. It must be emphasized, however, that the existence of salts of humic acid in the so-called fractionation of humus based on the solubility of these salts in one or the other solvent must be recognized. Just how misleading the results can be, will be clear from table 5, in which are given the solubilities of humic acid and of humates in water and alcohol. It is obvious that soils must contain humic acid and humates and that any attempt to determine the solubility of these in alcohol or water is bound to be misleading. A good deal of the earlier work based on the soluble and insoluble fractions in one or the other solvent must be revised in the light of these results.

The solubilities were determined by shaking 0.1 gram of humic acid and of humates with 20 cc. of water and alcohol (95 per cent) at room temperature (28–30 C.). No attempt was made to obtain absolute values. The values listed are merely intended to illustrate the magnitude of the differences that might be expected under average laboratory conditions. The results are expressed in terms of the percentage of humus gone into solution and obtained by filtration and titration of the filtrate by alkaline permanganate; 100 per cent, therefore, does not represent the upper limit of solubility.

SUMMARY

The formation of humic acid and of humates has been studied by following the changes in the hydrogen-ion concentration of the reaction media.

The complete precipitation of humic acid and of insoluble humates from sodium humate takes place when all of the sodium in the sodium humate has been replaced. The bearing of this phenomenon on the lack of stoichiometric proportion in humates is discussed.

PRELIMINARY PROGRAM OF MEETINGS OF THIRD COMMISSION ON SOIL MICROBIOLOGY OF INTERNATIONAL SOCIETY OF SOIL SCIENCE

The preliminary meetings of the Third Commission on Soil Microbiology of the International Society of Soil Science will be held in New Brunswick, New Jersey, on Wednesday, August 30, 1939. The meetings will last until Saturday, September 2, the date of the beginning of the Third International Microbiological Congress which is to be held in New York City. The meetings of the Commission on Soil Microbiology are arranged in cooperation with Section VIII on Agricultural and Industrial Microbiology of the Microbiological Congress.

The following subjects will be considered at the meeting of the Third Commission: 1. Legume Bacteria; 2. Microbiology of Organic Matter Decomposition; 3. The Soil Population. Titles of papers to be presented before this Commission should be submitted not later than July 1, 1938. The complete papers should be sent in before January 1, 1939. It is hoped that these papers will be published, in a volume of proceedings, before the meetings.

All correspondence concerning these meetings should be addressed either to Dr. H. G. Thornton, Rothamsted Experimental Station, Harpenden, England, or to Dr. S. A. Waksman, New Jersey Agricultural Experiment Station, New Brunswick, New Jersey.

THE PRESENCE OF ALLANTOIN IN SOILS

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U. S. Department of Agriculture

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From time to time since 1908 reports have been made by workers in the Division of Soil Fertility Investigations regarding the isolation and identification of organic soil constituents (2, 4, 5, 6, 7). For the most part such isolations have been made from an alkaline extract of soil, supplemented in a few instances by isolation of the same compound from an aqueous or alcoholic extract.

It has been argued that because of the possibility of the isolated compound's having been formed by chemical action of the alkali the compound may not be a true soil constituent. Although this possibility does obtain for some compounds, it would seem that the criterion for determining whether a specific compound could be formed by the treatment to which the soil has been subjected should be the known constitution and properties of the compound. Even when such formation by the alkali seems probable, the presence of some closely related antecedent compound is indicated.

Whatever may be the concentration and composition of the soil solution, it seems fair to assume that any organic compound present in a water extract of a soil must be present in some quantity in the actual soil solution and must be taken into consideration in any study of the effect of this solution on plant growth.

Although the isolation of organic compounds from a water extract of a soil would seem to be conclusive evidence of the presence of such compounds in the soil, the small quantity of organic matter in an aqueous soil extract has heretofore seemed such a serious obstacle to such isolation that until recently little concerted effort has been made to study the organic composition of an aqueous extract. It has been found, however, that the difficulties involved have been exaggerated, that it is possible to isolate and identify some organic compounds from a water extract of quantities of soil as small as 1 kgm., that for purposes of isolation a water extract has some advantages over an alkaline extract.

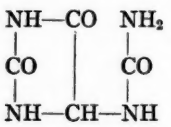
A number of soils of widely different types and composition have been studied in this connection. The method of obtaining a water extract was as follows. About 1 kgm. of air-dried soil in its natural field condition, except for the removal of sticks and stones, was placed in a glass percolator with

[1] Collaborator, formerly senior biochemist, Bureau of Plant Industry, Division of Soil Fertility Investigations.

nearly straight sides, the percolator being of such size that the soil came within 2 or 3 inches of the top. Distilled water, either at room temperature or heated not above 80°C., was poured on the soil, and the percolator was kept filled until 4 or 5 liters of extract had been obtained. If an extract of a larger quantity of soil was desired it was found preferable to obtain an extract from 1-kgm. units, rather than attempt to extract a larger quantity at one operation. With the soils so far used, no difficulty has been encountered in obtaining a clear extract of 4 or 5 liters in a few hours, but it is realized that there are some soils to which this method would not be applicable.

The after treatment of this extract has depended on the nature of the compound or compounds sought or suspected to be present. For the most part, precipitation with metallic salts such as lead acetate, silver nitrate, and mercuric nitrate, has been resorted to. In some cases this treatment was carried out with the original extract; in other cases, the extract was concentrated to smaller volume.

Among the organic compounds isolated from a water extract of soil, allantoin has been identified. Allantoin, $C_4H_6N_4O_3$, glyoxyldiureide, usually represented structurally thus,



has been isolated from four soils—Caribou loam from Maine; Norfolk sand from Columbia, South Carolina; Norfolk fine sand from Gunston Hall, Virginia; and a so-called Glade soil from Florida. The samples were from soils in cultivation, taken at an average plow depth.

The water extract obtained as described was treated with a solution of mercuric nitrate. This produced a flocculent precipitate, removing the slight yellow color of the extract. An excess of the reagent was then added, and the precipitate was removed by decantation or filtration. To the filtrate, a dilute solution of sodium hydroxide was added until the white precipitate first formed became yellow, the solution being still acid. Both precipitates, after being washed, were suspended in water, heated to boiling, and treated with hydrogen sulfide to complete precipitation of the mercury as sulfide, and the filtrates from sulfide were evaporated almost to dryness on a steam bath. The residue so obtained was partly crystalline, although that from the first precipitate contained considerable amorphous colored material, which apparently prevented, or made difficult crystallization. This partly crystalline material was washed well with absolute alcohol, taken up in hot water, filtered, and evaporated again to the crystallizing point. This operation was repeated several times, until finally crystals that had the appearance of allantoin deposited on cooling of the hot solution.

The appearance of these crystals is usually stated in the literature as characteristic, and photomicrographs of them have been published. Although it is true the crystals have a characteristic appearance, this alone is of course not sufficient for identification. Photomicrographs of allantoin from Caribou loam are shown in plate 1, figures 1, 2, and 3. A photomicrograph of pure allantoin purchased in the market is shown in plate 1, figure 4.

In all precipitations of soil solutions or extracts with salts of lead, mercury, or silver, calcium sulfate if present, as it is in nearly all soil solutions, is carried down and appears in the filtrate after treatment with hydrogen sulfide. This calcium sulfate, which crystallizes readily and which occurs in many filtrates in quantities in excess of the organic material, presents one of the difficulties in obtaining any organic soil compound in a pure form. Inasmuch as allantoin, however, is almost insoluble in alcohol, very slightly soluble in cold water, and very soluble in hot water, whereas calcium sulfate is somewhat less soluble in hot water than in cold, the treatment outlined above results in obtaining allantoin in a pure form, if sufficient is present in the original precipitate. The procedure necessarily involves considerable loss of allantoin, probably 50 per cent of that in the original crystalline residue.

The allantoin obtained from the soils mentioned has been identified by the following properties:

Solubility. Insoluble in ether and very difficultly soluble in alcohol. Difficultly soluble in cold water but very soluble in hot.

Decomposition on heating with mineral acids. Under this treatment allantoin yields urea as one of the products. This has been identified by precipitation with xanthydrol.

Decomposition on heating with concentrated solution of sodium hydroxide. Oxalic acid is one of the products from this treatment and has been identified by precipitation as calcium oxalate.

General properties. Odorless and tasteless. Precipitated by mercuric salts. Browns at a low temperature and melts with decomposition at about 240°C. Reaction—stated by some authorities to be neutral to litmus and by others to be faintly acid; the pH of a commercial product was found to be 6.7.

Schiff's reaction. A few drops of a concentrated aqueous solution of furfurol added to a small crystal of allantoin followed by a few drops of concentrated HCl produces a yellow color which turns purple. The solution of furfurol must be freshly prepared. A similar color is given by urea and cyanuric acid, but either of these compounds can readily be distinguished from allantoin by other properties. This reaction is very delicate and indicates mere traces of allantoin. Contamination with calcium sulfate does not interfere.

Nitrogen content. The theoretical nitrogen content of allantoin is 35.4 per cent. Sufficient material for a micro-kjeldahl nitrogen determination has been obtained from only one soil, where duplicate determinations gave 35.0 and 35.3 per cent.

In obtaining the water extract the percolation was continued until mercuric nitrate no longer gave a precipitate; therefore, it may be assumed that all the soluble allantoin was removed. If allowance is made for as much as 50 per cent loss in purification, the quantity present in the soils examined is indicated as small, a few milligrams per kilo, or a few parts per million. Allantoin has been found in a number of plants and in animal tissues and fluids;

it is now regarded as a constant product of the breaking down of nucleoproteids; and it is probably present in many places where it has not been noted, simply because it has not been looked for.

Allantoin has been reported to be a cell proliferant, but this has been disputed by some investigators. To determine whether or not it had an effect on the rooting of cuttings, a number of *Coleus* cuttings were rooted in both sand and distilled water, with and without allantoin in solution. No differences could be detected in the number or appearance of rootlets developed. In water containing 0.05 per cent allantoin, however, although many rootlets developed, the leaves after 2 weeks, showed an efflorescence which was identified as crystals of allantoin. In this solution, the leaves turned brown, and after 3 weeks the cuttings were dead.

Several years ago a number of organic compounds were tested in this laboratory for possible toxicity to wheat seedlings (3). Among the compounds, allantoin was tested, but no toxicity was indicated. The nature of this experiment was such that it furnished no data bearing on the utilization of allantoin as a supply of nitrogen.

Allantoin has recently received considerable notice because it was found that maggots used for treating wounds slow in healing, secreted allantoin (1). With this in mind, earthworms were examined for the presence of allantoin. A few grams of dried earthworms, extracted with water and subjected to the treatment outlined, yielded sufficient allantoin for conclusive identification. Because of the known activity of earthworms in some soils, it might be assumed that allantoin might arise from this source in the soil, but since it was also found in a soil (sand) in which earthworms were not likely to be active, it does not seem necessary to attribute the presence of allantoin to this source.

Since allantoin yields both urea and ammonia rather readily, it seems fair to assume that this compound is a source of supply of nitrogen for plant nutrition, even though it may not be utilized as such directly.

SUMMARY

Allantoin, an organic nitrogenous compound, has been isolated from the water extract of four soils of different type and composition. The quantity isolated has been small—a few parts per million—probably equal to the average nitrate content of cultivated soils. It has been shown that it is possible to isolate and identify an organic soil constituent from an aqueous extract of 1 kgm. of soil.

Whether or not allantoin can be directly assimilated by growing plants is not yet known, but since it is rather easily transformed into urea or ammonia, it must be considered as part of the soil nitrogen available for plant growth.

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PLATE 1

PHOTOMICROGRAPHS OF ALLANTOIN

FIGS. 1, 2 AND 3. Allantoin from Caribou loam
FIG. 4. Pure allantoin purchased in the market

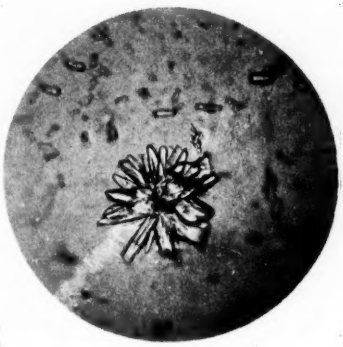

FIG. 1

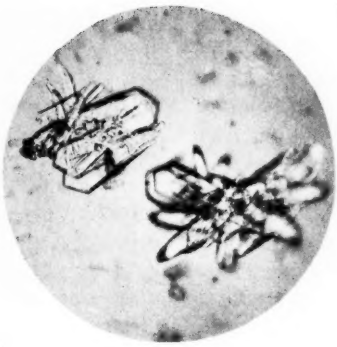

FIG. 2

FIG. 3

FIG. 4

IDENTIFICATION OF PHYTOMONAS, AZOTOBACTER, AND RHIZOBIUM OR ACHROMOBACTER UPON INITIAL ISOLATION*

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A large number of media have been suggested as being desirable for the growth of *Rhizobium* and *Azotobacter*. Of these, the nitrogen-free medium of Ashby (1) is satisfactory. In isolating or making plate counts of *Rhizobium* or *Azotobacter* from the soil, *Phytomonas tumefaciens* and *Achromobacter radiobacter* are frequent contaminants, thereby decreasing the accuracy of such determinations. Furthermore, it is extremely hard, if not impossible, to differentiate colonies of *Rhizobium* from *Azotobacter* in Ashby's medium.

In 1911 Kellerman (4) reported the use of congo red in a mannitol-nitrate medium to differentiate colonies of *Rhizobium* and *Phytomonas*; the *Phytomonas* colonies assumed a deep red color, and the *Rhizobium* colonies remained colorless. Other investigators reported little difference in the adsorption of the dye by *Phytomonas*, *Rhizobium*, and *Achromobacter*. Fred, et al. (2) list a medium containing gentian violet, which was suggested by Leonard (5) in 1931, in which the *Rhizobium* colonies are small and colored throughout the *Achromobacter* colonies have deep blue or violet centers with clear rims. Although surface colonies give these color and size differences, subsurface colonies of both organisms are small and colored throughout.

A study of the adsorption of congo red by *Rhizobium*, *Achromobacter*, *Phytomonas*, and *Azotobacter* when grown in a nitrogen-free medium containing the dye is desirable in view of the fact that any or all of these microorganisms, if present in the original sample, may develop on the nitrogen-free agar.

METHOD

Pure cultures of bacteria that grow on nitrogen-free agar were studied with respect to their adsorption of congo red present in the medium. These included 15 strains of *Azotobacter*, 15 strains of *Phytomonas*, 10 strains of *Achromobacter*, and 30 strains of *Rhizobium*. The strains of *Rhizobium* were recently isolated from fresh nodules, the *Phytomonas* were recently checked for pathogenicity, the *Azotobacter* were recently isolated from the soil and checked

* Journal Article No. 298, New Series, from the Michigan Agricultural Experiment Station, section of bacteriology.

for their ability to fix nitrogen non-symbiotically, and *Achromobacter* were obtained from A. W. Hofer, of New York. Numerous soils and macerated nodules were plated in Ashby's medium containing 10 cc. of a 1-400 dilution of Congo red (1-40,000 final dilution of dye in the medium) per liter of agar. Later 1-20,000 final dilution of the dye in the medium was used. The plates were incubated at room temperature and examined daily to determine the time necessary for maximum adsorption of the congo red. The cultures isolated were further identified by biochemical tests.

RESULTS AND DISCUSSION

Variations in adsorption of the dye became evident at the fourth day of incubation, maximum adsorption of the congo red by the subsurface colonies occurring in 7 to 10 days. The color of the subsurface colonies was: *Achromobacter* and *Rhizobium* white, *Azotobacter* pink, and *Phytomonas* red. Two of the *Azotobacter* colonies were a faint pink and could readily be confused with the *Achromobacter* or *Rhizobium* colonies. In order to obtain better differentiation the congo red was doubled in amount by adding 1-20,000 final dilution to the agar. This had the desired effect, for the subsurface colonies of *Phytomonas* were red, *Azotobacter* pink, and *Achromobacter* and *Rhizobium* white. The surface colonies did not adsorb the dye as did the subsurface colonies. This suggested the practice of capping the plates so that all colonies would develop as subsurface colonies.

Several types of materials are available for capping the plates. Paraffin which ordinarily does not support the growth of bacteria is usable but undesirable because of its messiness. The nitrogen-free agar without congo red gave good results in capping plates but does not have any virtues above those of the congo red nitrogen-free agar which is recommended for culturing the bacteria that grow on the nitrogen-free medium. Incubation at room temperature for 7 to 10 days permitted maximum differentiation of the colonies.

The color developed permits identification of *Phytomonas* and *Azotobacter* colonies but does not differentiate between *Rhizobium* and *Achromobacter*, a differentiation that is valuable when one is making isolations and plate counts of the specific microorganisms. In order to determine the count of *Rhizobium* and *Achromobacter*, duplicate plates are made in a nitrogen-free medium of pH 11, as suggested by Hofer (3). The *Achromobacter* alone grow in this medium. This *Achromobacter* count subtracted from the total count of white colonies in the congo red medium gives the *Rhizobium* count.

Colony formation is essential for proper identification, since streak inoculations did not permit adequate adsorption of the dye to give differentiation.

SUMMARY

Because of the presence of *Achromobacter* and *Phytomonas* colonies, much confusion results in the determination of the *Azotobacter* content of the soil and in the isolation of *Rhizobium* from nodules. The addition of 20 cc. of a

1-400 aqueous solution of congo red (1-20,000 final dilution in the medium) affords differentiation of *Phytomonas*, *Azotobacter*, and *Rhizobium* or *Achromobacter* colonies. The plates must be capped with the same medium to insure all colonies developing as subsurface colonies. The subsurface colonies of *Phytomonas* are red, those of *Azotobacter* are pink, and those of *Rhizobium* and *Achromobacter* are white. Duplicate dilutions should be plated in a nitrogen-free agar of pH 11; only *Achromobacter* grow in this medium. This plating yields data with respect to count of *Rhizobium* and *Achromobacter* and serves as a means of differentiating between these two organisms.

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IMPORTANCE OF SILICON, ALUMINUM, AND CHLORINE FOR HIGHER PLANTS

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During the last decade or more, we have experimented in this laboratory with the problem of some of the chemical elements which, until a few years ago, were regarded as nonessential to higher plants. As a result of our experiments, we have demonstrated initially or have confirmed the results of others respecting the essential nature of Cu, Zn, and B. These experiments have been reported in papers[1] published from time to time. We have, however, carried out experiments with other chemical elements upon which we have made only incomplete reports[2] or no reports at all. In this work, special emphasis has been placed on the question of whether or not Al, Si, and Cl are essential to the life and growth of higher plants. Our answer, and that of others, concerning the indispensable nature of Cu, Zn, and B has been unequivocal, and we have furnished proof of the indispensable nature of those elements to several of the higher plants. We have found, in common with other investigators, that it has thus far not been feasible to prove that Al, Si, and Cl, however, are indispensable, in the strict sense of the term, for higher plants. On the other hand, our results seem to indicate that those three elements are distinctly beneficial to the growth of certain plants with which we have worked, and I propose to submit the evidence herewith.

These experiments were carried out in culture solutions in Mason jar containers, except as otherwise indicated. The plants were grown to maturity. The solutions and the technic used, which varied somewhat from series to series in the experiments, are described separately under each experiment.

EXPERIMENT I—SUNFLOWERS GROWN WITH AND WITHOUT SILICON

Sunflower plants were grown from germinated seedlings in 2-quart Mason jars which were coated inside with Valspar. Only one plant was placed in each jar. The solution used was constituted as follows:

KNO_3	0.8 gm. per liter
$MgSO_4 \cdot 7H_2O$	0.5 gm. per liter

[1] SOMMER, A. L., AND LIPMAN, C. B. 1926 Evidence on indispensable nature of zinc and boron for higher green plants. *Plant Physiol.* 1: 3.

LIPMAN, C. B., AND MACKINNEY, G. 1931 Proof of the essential nature of copper for higher green plants. *Plant Physiol.* 6: 593–599.

[2] SOMMER, A. L. 1926 Studies concerning essential nature of aluminum and silicon for plant growth. *Univ. Calif. Publ., Agr. Sci.* 5: 2.

KH_2PO_4	0.15	gm. per liter
$CaSO_4$ saturated solution	300	cc. per liter
B as H_3BO_3	0.5	p.p.m.
Cl	5	p.p.m.
Na	5	p.p.m.
Mn	2	p.p.m.
Al	1	p.p.m.

This culture solution was used in 16 jars, and the same solution to which was added silicon in the form of freshly prepared colloidal silicon dioxide in pure form was used in 16 additional jars. The culture solutions were aerated.

TABLE 1

Yields of dry matter in sunflower plants grown with and without silicon

CULTURE NO.	WITHOUT Si		WITH Si	
	Total	Tops alone	Total	Tops alone
	gm.	*gm.*	*gm.*	*gm.*
1	2.5	1.8	2.6	1.8
2	2.0	1.5	7.0	4.0
3	2.1	1.5	7.3	5.1
4	3.3	2.2	6.1	4.6
5	2.5	1.9	5.8	4.1
6	3.3	2.4	4.4	3.6
7	2.0	1.4	3.8	2.6
8	4.4	3.3	3.9	2.9
9	3.7	3.0	8.6	5.8
10	2.6	2.0	2.0	1.4
11	2.7	1.9	5.8	4.1
12	1.7	1.3	2.9	2.3
13	7.8	5.9	5.1	4.1
14	2.8	2.2	6.5	4.9
15	2.0	1.4	9.2	7.0
16	2.3	1.6	5.3	3.8

The experiment was started on April 7. The plants were harvested on June 5. The results are given in table 1, which shows the yields of dry matter of each culture as a whole and of the tops alone.

Making due allowance for the variability in the yield of individual plants which is obvious in table 1, one cannot help but be struck by the vastly better yields obtained when silicon is present in the culture solution than when it was absent. Another feature of the experiment which helps perhaps to account for the variability in the yields is the coat of Valspar on the inside of the jars which must have contributed irregularly small quantities of silicon. The salts used in the culture solution were very pure, and the Valspar coating was intended to prevent solution of silicon from the glass of the jar. All in all, the effect of silicon on the growth of sunflowers is remarkable when one

considers that there must have been some silicon in the Valspar, a trace in the chemicals, and an appreciable amount in the seedlings when they were started. It is not unlikely, therefore, that if these sources of silicon could be removed from the experiment, very little growth of the seedlings would have been obtained in the 16 control jars.

EXPERIMENT II—BARLEY GROWN WITH AND WITHOUT SILICON

The culture solution used in experiment II was the same as that in experiment I, except that 1.5 p.p.m. of Mn was used instead of 2 p.p.m., and 0.25 p.p.m. each of Cu, F, and I, 12.7 p.p.m. NaCl, and 0.5 p.p.m. Al were added.

Instead of Mason jars, 1-liter Pyrex beakers were used as containers for the culture solution. Four barley seedlings were used per beaker; the seed

TABLE 2

Yields of barley as dry matter, grown with and without silicon

CULTURE NO.	WITHOUT Si				WITH Si			
	Tops minus heads	Heads	Roots	Total	Tops minus heads	Heads	Roots	Total
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	39.9	4.7	4.0	48.6	48.2	8.3	6.3	62.8
2	30.6	6.9	3.2	40.7	37.6	3.0	3.6	44.2
3	31.3	1.5	3.0	35.8	42.2	3.4	6.3	51.9
4	20.0	0.2	3.0	23.2	26.6	3.9	3.8	34.3
5	28.4	1.0	3.1	32.5	38.7	8.4	4.0	51.1
6	36.1	2.5	3.3	41.9	31.0	6.1	2.7	39.8
7	32.5	0.7	3.8	37.0	34.5	6.5	3.7	44.7
8	25.3	0.9	2.7	28.9	35.7	2.9	3.0	41.6
9	18.6	1.1	2.7	22.4	42.9	4.9	5.1	52.9
10	33.2	3.7	3.1	40.0	42.4	10.2	4.0	56.6

coat and the stored seed food were removed; and the seedlings were set out in the one-leaf stage. Ten beakers were used for the control plants and ten for those receiving silicon dioxide. The culture solutions were changed from time to time, or additions of important ingredients like KNO_3 and $MgSO_4 \cdot 7H_2O$ were made as seemed necessary. The experiment was started on December 1, and the plants were harvested on April 20. In February, a tendency in some cultures for the roots to blacken because of bacterial decomposition was checked by aeration, which resulted in marked improvement of the roots. The results of the experiment are given in table 2.

It is perfectly clear from the data of table 2 that the barley plants supplied with silicon possessed a great advantage over those grown in culture solution without silicon but under otherwise identical conditions. If the values for dry matter yields of tops, heads, or roots, and particularly the first two, are

arranged in ascending or descending order, it becomes strikingly evident that in some way the presence of silicon confers upon the barley plant a capacity for growth which far outdistances that of the plant not furnished any silicon. This is true in spite of the obvious variability which characterizes the individual cultures and is all the more impressive when one observes that the plants not furnished any silicon still possessed some in the seedling, in the chemicals, in the glass beakers, and in dust from the air in the greenhouse compartment in which the cultures were grown.

EXPERIMENT III—SUNFLOWERS GROWN WITH AND WITHOUT ALUMINUM

The culture solution employed in this experiment, was essentially the same as the one used in experiment II. Pyrex beakers of about 1 liter capacity were used as containers. Three sunflower seedlings were grown in each beaker. Sixteen beakers were used containing the basic culture solution plus 1 p.p.m. Al as aluminum sulfate, and 16 beakers, as controls with the same culture solution but no addition of aluminum. The plants were set out on June 12 and harvested on October 15. The solutions were changed seven times during the experiment either completely or by additions of certain individual salts like KNO_3 when there were indications that the latter was required.

Since the plants in the two groups were of about the same size, no dry weight determinations were made in this series, but the weight of the heads was determined. The plants receiving aluminum produced 23.3 gm. of heads, whereas those not receiving aluminum yielded only 20.9 gm. The lack of such marked effects as those given in the silicon series preceding renders it unnecessary to discuss the results in more detail here, especially since the next experiment with maize gave much more striking results and will be discussed next.

EXPERIMENT IV—MAIZE GROWN WITH AND WITHOUT ALUMINUM

The series with maize was prepared with the same solution as that used in experiment II, but 2-liter Mason jars were used as containers in place of the Pyrex beakers. The maize seed was germinated, and in the two-leaf stage one seedling was transferred to each culture jar. Twenty-nine cultures each with an addition of 1 p.p.m. Al as aluminum sulfate and twenty-nine receiving no Al but otherwise the same were employed. Additions of salt were made to the cultures from time to time as seemed desirable, the water lost by transpiration being replaced when necessary. Tassels began to appear about 1 month after the commencement of the experiment. In about 6 weeks it became apparent that the plants receiving Al were not only larger than the controls but were showing more heads, which were developing corn silk much more generally. For example, on October 7, the plants receiving Al had 36 ears, 14 of which showed silk, and those receiving no Al had only 21 ears, only 2 of which were showing silk. On October 16, the corresponding

figures were 37 and 15 for the plants receiving Al, and 25 and 7 for the plants not receiving Al. About October 20, all the plants suffered from injury by excessive heat. The yields of total dry matter and of dry matter in the ears alone are given in table 3. The values are arranged in regular descending order in each series.

TABLE 3

Yields of total dry matter and of dry matter in ears of maize grown with and without aluminum

TOTAL DRY MATTER		DRY WEIGHT OF EARS	
With Al	Without Al	With Al	Without Al
gm.	*gm.*	*gm.*	*gm.*
28.6	21.2	3.5	1.1
26.0	20.0	2.4	0.8
24.9	18.8	1.7	0.7
23.5	18.6	1.5	0.6
21.8	18.6	1.2	0.6
21.3	17.9	1.2	0.4
21.3	17.7	1.1	0.4
21.2	17.4	1.1	0.4
20.6	17.1	1.1	0.4
20.6	17.1	1.1	0.4
19.6	16.5	1.0	0.3
19.4	16.1	0.9	0.3
18.9	16.0	0.9	0.3
18.0	15.5	0.7	0.3
18.0	15.4	0.7	0.3
17.9	15.3	0.6	0.3
17.2	15.1	0.5	0.3
16.5	15.0	0.4	0.2
16.5	13.3	0.4	0.2
16.4	13.3	0.3	0.2
16.0	13.2	0.3	0.2
14.3	13.2	0.2	0.1
13.9	12.9	0.2	0.1
13.5	12.9	0.1	0.1
13.5	12.7	0.1	0.1
13.4	12.0		
13.1	9.5		
11.4	9.3		
9.7	8.5		
Total 527.0	440.1	23.2	9.1

In summing up the results given in table 3, it is very impressive to note that not only were the individual plants of corn receiving Al superior, in general, to those not receiving Al, but the total yield of dry matter was 527 gm. in the cultures receiving Al and only 440.1 in those not receiving Al. In addition, the plants receiving Al produced a total of 40 ears weighing 23.2 gm., whereas

those not receiving Al produced 33 ears which weighed 9.1 gm. When all due allowance is made for variability in individual cultures, therefore, and for such other factors as those discussed under experiment II, which militate against obtaining a full picture of the actual behavior of the plants in the presence or absence of a given indispensable chemical element, there can hardly be any doubt that in this case again, to put it very conservatively, Al plays a rôle of great importance in the life of such higher plants as corn and sunflowers and probably of many others.

EXPERIMENT V—BUCKWHEAT GROWN WITH AND WITHOUT CHLORINE

Conflicting results reported in a fairly voluminous literature on the importance or nonimportance of chlorine to plants rendered it desirable to make further experiments concerning this question. Buckwheat was chosen as the experimental plant because a number of other investigators had worked with it in connection with the same problem and because the conflicting results obtained therewith seemed peculiarly puzzling. A preliminary experiment in which the plants were injured through some abnormality on the roots indicated that plants receiving additions of chlorine to the culture solution were able to produce more and larger flowers and more seed than those receiving no chlorine. Hence the experiment was repeated. The following culture solution was used:

KNO_3	0.6 gm. per liter
KH_2PO_4	0.1 gm. per liter
$MgSO_4 \cdot 7H_2O$	0.3 gm. per liter
$CaSO_4$ saturated solution	300 cc. per liter
Mn	2 p.p.m.
Al	0.5 p.p.m.
B	0.5 p.p.m.

Because of the difficulty experienced in the preceding experiment just cited, all solutions, corks, and cotton were sterilized. The seed was treated with $HgCl_2$ (1:5,000) for 10 minutes. The seedlings were protected in a sterile jar while germinating. Eight cultures were set up in Mason jars with five seedlings per culture and with chlorine added at the rate of 5 p.p.m. in the form of pure KCl. Eight additional cultures were set up as controls, everything about them being the same as in the first eight cultures except that they received no chlorine. The solutions were changed from time to time. The plants were set out on May 4 and harvested on July 20. Toward the end of the experiment, all plants suffered from sunburn. Since the total dry weights for the controls and for the chlorine-treated plants were not appreciably different but a marked difference existed between the two as regards the weight of seeds produced, we give here only the weight of the seeds as follows:

Plants receiving chlorine produced 11.2 gm. seeds
Plants not receiving chlorine produced 7.3 gm. seeds

It seemed desirable to repeat this experiment once more, this time employing seeds from the harvest just described. In other words, it seemed desirable not only to determine whether the marked seed discrepancy just demonstrated was of any significance, but also whether or not the two sets of seeds produced in the foregoing experiment were so affected by the treatment which their parents had received as to show an accentuation of the effects produced by chlorine in the growth of buckwheat as compared with the first generation. Accordingly, experiment VI was inaugurated.

EXPERIMENT VI—SEED OF BUCKWHEAT PRODUCED IN EXPERIMENT V, GROWN WITH AND WITHOUT CHLORINE

The culture solution employed in experiment VI was the same as that for experiment V except that 0.5 p.p.m. Zn and 0.25 p.p.m. Cu were added.

TABLE 4

*Yields of dry matter, per jar, of tops, roots, and seeds of buckwheat from second generation grown with and without chlorine**

CULTURE NO.	WITH CHLORINE			WITHOUT CHLORINE		
	Tops	Roots	Seeds	Tops	Roots	Seeds
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	4.80	0.50	0.90	1.05	0.15	0.00
2	4.10	0.50	0.45	2.50	0.40	0.20
3	3.60	0.40	0.60	1.35	0.25	0.05
4	2.55	0.35	0.00	0.55	0.05	0.05
5	2.60	0.30	0.20	1.20	0.25	0.05
6	2.20	0.30	0.20	0.70	0.15	0.05
7	1.85	0.25	0.15	2.15	0.30	0.15

* In the series with chlorine, all four seedlings in each jar grew and matured except one seedling in culture 7 which was broken when very young, and hence the lowest dry matter value of the series is in that culture. In the series without chlorine, only three plants survived; only 2 plants survived in culture 4; and only 3 plants survived in cultures 5 and 6.

Seven jars carried seedlings from seeds produced with chlorine additions in experiment V, and seven others, the seedlings from seeds produced in the same experiment without additions of chlorine to the culture solution. Where chlorine was administered, it was as before at the rate of 5 p.p.m. of Cl as KCl. Of the two groups of seeds of the special history just given, those produced without added Cl showed a germination of only 51 seeds, whereas those produced with additions of chlorine gave a total of 131 germinated seeds. Of these germinated seeds, only 28 seedlings developed in the first group, whereas nearly all developed in the second group. The seedlings were placed in the jars on January 30, some of the control jars not having a full complement of seedlings for the reason just given. Salts were added to culture solutions from time to time as required. During the growing period, the control

plants were highly variable, some individuals being very weak and others about as good as some of those receiving chlorine. The plants receiving chlorine began producing seeds several days earlier than the controls. They were all harvested on April 15. The yields of tops, roots, and seeds in all cultures are given in table 4.

In spite of the fact that the total growth produced in these cultures was not very great, it is clear that the plants receiving chlorine were, in general, far superior to those not receiving that element. It is notable that the plants receiving chlorine produced 2.50 gm. of seed as against 0.55 gm. for the plants not receiving chlorine. It is particularly interesting to note the exaggerated weakness in all respects of the plants not receiving chlorine, which must be explicable on the ground that the seeds from which they grew started with the handicap of being progeny of plants grown without additions of chlorine to the medium. When the evidence given in experiment V and that given in experiment VI are taken together, it is difficult to avoid the conclusion that if chlorine is not absolutely essential to the buckwheat plant, it certainly is very important to it in some way which enables it to make better growth and especially to produce more seed than it would without that element.

EXPERIMENT VII—PEAS GROWN WITH AND WITHOUT CHLORINE

In the series in which peas were grown with and without chlorine, two experiments were carried out because the roots of the plants were injured by fungi in the first experiment and the growth made by all plants was unsatisfactory. The seed harvested from this first attempt in this series, nevertheless, showed a yield of 21.1 gm. for the plants grown with chlorine and only 18.1 gm. for those grown without chlorine. The seed thus obtained was used for a repetition of the experiment. The culture solution was as follows:

KNO_3	0.8	gm. per liter
$CaSO_4$ saturated solution	300	cc. per liter
$MgHPO_4$	230	p.p.m.

The usual additions in small quantities were made, as in the preceding series, of the following elements, Al, B, Zn, Cu, F, I. Twelve 2-quart Mason jars were used as containers for plants receiving no Cl, and 12 similar jars were used for plants receiving additions of Cl, which were made at the rate of 5 p.p.m. Cl in the form of KCl. Four pea seedlings were planted in each jar. The experiment was started on January 19, and the plants were harvested on April 8. At the beginning of the experiment, the seedlings grown from seed whose parents had received chlorine showed better top development and much better root development than did those grown from seed whose parents had received no chlorine. The yields of tops, roots, and seeds per jar are given in table 5.

No appreciable difference is evident in table 5 in the yields of dry matter of tops, roots, or seeds of the pea plants of the second generation as between

those receiving Cl and those not receiving it in spite of the promise of distinct difference at the beginning of the experiment to which reference has been made. Accordingly, the seeds of each series were saved for a third generation experiment. An opportunity to carry out this additional trial did not present itself for several years, during which the seeds were preserved in test tubes with cotton stoppers. Last year the additional experiment was carried out with the following results: In appearance the plants without Cl were slightly but not much inferior to those with Cl, but a very marked difference was apparent in the yield of seeds. From the plants receiving chlorine, 99 seeds were obtained having a weight of 18.9 gm., whereas from the plants not receiving chlorine only 77 seeds were obtained having a weight of 10.0 gm. More-

TABLE 5

Yields of dry matter, per jar, of tops, roots, and seeds of peas from second generation grown with and without chlorine

CULTURE NO.	WITH CHLORINE			WITHOUT CHLORINE		
	Tops	Roots	Seeds	Tops	Roots	Seeds
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	4.40	0.65	1.30	2.47	0.55	1.45
2	4.95	0.63	2.40	6.25	0.70	3.10
3	4.95	0.60	2.40	4.45	0.50	2.00
4	5.85	0.70	2.35	5.00	0.60	1.80
5	5.15	0.55	2.10	5.50	0.70	2.45
6	5.70	0.60	2.65	4.45	0.50	2.10
7	6.10	0.60	2.65	5.70	0.70	2.45
8	5.55	0.65	2.10	5.25	0.75	2.70
9	4.95	0.55	2.25	5.80	0.70	2.70
10	4.35	0.50	1.85	5.05	0.70	2.05
11	5.55	0.50	2.35	4.20	0.60	1.45
12	4.70	0.45	2.25	5.20	0.80	1.75

over, the seeds from the +Cl series are plump and large, whereas those from the −Cl series are small and shriveled. When all allowances are made for the difficulties and uncertainties of the experiments with peas in the chlorine series, it seems to be definitely indicated that chlorine performs an important function in peas, as it does in buckwheat, and particularly so in connection with seed formation.

SUMMARY

In experiments to ascertain the importance or nonimportance of Si, Al, and Cl for several higher plants grown in culture solutions, the following results were obtained:

Sunflowers and barley are definitely benefited, especially as regards seed production, by the presence of Si in the culture medium.

Sunflowers and maize are definitely benefited, especially as regards seed production, by the presence of Al in the culture medium.

Buckwheat and peas are definitely benefited by the presence of Cl in the culture medium. With buckwheat, this is true for both total dry matter production and for seed production. With peas, it seems to be true, on limited experiments, only for seed production.

The probability that all three elements may be indispensable to the higher plant is very great indeed, but these experiments leave that question open. They do prove the importance of Si, Al, and Cl for the plants investigated and probably for all higher plants. It is highly desirable that further experiments be conducted with still greater control as regards supply of the elements under study.

A METHOD FOR STUDYING DECOMPOSITION OF ISOLATED LIGNIN, AND THE INFLUENCE OF LIGNIN ON CELLULOSE DECOMPOSITION[1]

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It has been definitely established (1, 2, 3, 10) that, among the plant constituents, the lignin is more resistant to decomposition than are the other compounds. This resistance is not absolute, however, especially under aerobic conditions. When plant residues and stable manures undergo decomposition in composts and in soil, lignin is also attacked, although at a slower rate than are the carbohydrates and proteins (6, 7, 9). When lignin is isolated from plant material by one of the different chemical procedures, it does not seem to be attacked at all by microorganisms.

The resistance of isolated lignin to decomposition has been assumed to be due to a change in the chemical nature of the lignin in the process of its preparation from the plant material. This tends to give weight to the idea of certain chemists that isolated lignin is chemically different from its precursor in plant tissues. That this is probably not the sole explanation can be substantiated by the following two facts: 1. When wood is attacked by the "brown rot" fungus the cellulose is destroyed, whereas the lignin is left; this lignin is also resistant to decomposition by microorganisms. 2. It has been shown elsewhere (8) that when phenol-lignin or alkali-lignin is dispersed in a liquid medium, a certain amount of it is attacked by a variety of different organisms, including fungi and bacteria.

The aforementioned method of dispersion of lignin has a number of limitations, primarily because of the difficulty of removing all the alcohol that was used as a solvent for the lignin. Liquid media, furthermore, are not so satisfactory for the growth of many organisms as are solid media; the latter approach more nearly the physical conditions of a soil or a compost. Some of these difficulties were overcome by the use of the following procedure. A concentrated solution of lignin in ethyl alcohol was added to dry macerated filter paper as a source of cellulose; the alcohol was then completely removed by evaporation at a temperature of 60°C. The cellulose-lignin mixture was frequently stirred, in the process of evaporation of the alcohol, so as to coat the cellulose thoroughly with the lignin. A uniform product was thus obtained,

[1] Journal Series paper, New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

which can be designated as an artificial cellulose-lignin complex. This product was subsequently used for studies of the decomposition of lignin, by mixed populations and by pure cultures, in soil and in sand media. It was also used for comparative studies, with natural materials, on the influence of lignin on cellulose decomposition.

Both phenol-lignin and alkali-lignin were employed. Phenol-lignin, prepared from oat straw, spruce wood, or peat was dissolved in alcohol, and amounts equal to 20-gm. portions of the dry lignin were added to 50-gm. portions of ground filter paper. The preparations thus obtained gave, on hydrolysis with 80 per cent H_2SO_4, 60.4 per cent cellulose, calculated from the sugar produced, and 25.4 per cent lignin. Alkali-lignin was prepared by extracting oat straw with 4 per cent NaOH solution under pressure, precipitating with HCl, and washing. The lignin was treated with hot ethyl alcohol, whereby about half of it was made soluble; an equivalent of 20 gm. of lignin in solution was used for coating 50 gm. of cellulose by the aforementioned procedure.

Two-gram portions of the air-dry cellulose-lignin preparations and 100-gm. portions of washed sand were placed in 250-cc. Erlenmeyer flasks. Twenty cubic centimeters of water containing 100 mgm. $NaNO_3$, 100 mgm. K_2HPO_4, 50 mgm. $MgSO_4$, and a trace of $FeCl_3$ was added to each flask. The flasks were sterilized and inoculated with pure cultures of several organisms as well as with an infusion of fresh soil. For comparative purposes, a parallel series was prepared containing 1.5 gm. of cellulose in 100 gm. of sand and the aforementioned minerals.

The final analyses were made as follows: The residual organic matter was washed from the sand by means of distilled water. The washings were acidified with HCl and filtered through weighed papers. The residue was weighed, dried, and thoroughly treated with hot alcohol to extract the lignin. The latter was determined in the extract, by evaporation and ignition. The residue was analyzed for cellulose. In some cases, the total residue was hydrolyzed with cold 80 per cent H_2SO_4, diluted with water, and hydrolysis completed by heating; sugar and insoluble lignin were then determined.

The results of decomposition of alkali-lignin in the cellulose-lignin preparations, by pure and mixed cultures of organisms, for a period of 6 months at 28°C. are reported in table 1. Certain organisms, present in the complex soil population, especially among the fungi, were found to be able to decompose purified lignin. In addition to this important fact, the following facts were brought out in this experiment: 1. In the decomposition of cellulose, the organisms synthesize a small amount of ligninlike material; if allowance is made for the amount of lignin synthesized in the decomposition of the cellulose-lignin preparations the reduction in lignin may be found to be even greater than that shown in the table; 2. less nitrate was left and, therefore, assimilation of nitrogen by the organisms decomposing the cellulose in the presence of lignin was greater than that in its absence; 3. the presence of lignin had no injurious effect upon cellulose decomposition.

The decomposition of phenol-lignin in cellulose-lignin preparations by different organisms is shown in table 2. In this experiment, two sources of lignin were used, prepared from lowmoor peat and from straw composts. In this experiment, as well, extensive decomposition of both cellulose and lignin took place by different fungi and by a mixed fungus flora.

TABLE 1

Decomposition of cellulose and cellulose-lignin in sand media*

Milligrams per flask

ORGANISM	CELLULOSE ALONE				CELLULOSE-LIGNIN PREPARATION				
	Cellulose		Nitrate-N left	Lignin left†	Cellulose		Nitrate-N left	Lignin	
	Left	Decomposed			Left	Decomposed		Left	Decomposed
Control	1,523	0	15.13	10	1,010	0	14.45	456	0
Rhizopus sp.	1,301‡	222	11.50	17	1,037	0	14.50	456	0
Trichoderma sp.	1,209	314	7.96	24	633	377	1.57	448	8
Soil infusion	913	610	1.60	65	608	402	0.14	314	142
Fusarium sp.	1,260	263	9.69	34	637	377	Trace	322	134
Fungus No. 7	1,180	343	11.27	32	801	209	0.44	365	91

* Alkali lignin from straw used.

† Synthesized lignin and lignin impurities in cellulose.

‡ Culture became contaminated with *Trichoderma*.

TABLE 2

*Decomposition of phenol-lignin by fungi in cellulose-lignin preparations**

Milligrams per flask

ORGANISM	SOURCE OF LIGNIN	CELLULOSE		LIGNIN	
		Left	Decomposed	Left	Decomposed
Control	Peat	1,468	...	498	...
Coprinus radians	Peat	957	511	328	170
Fusarium sp.	Peat	1,221	247	332	166
Mixed fungus flora†	Peat	1,128	340	326	172
Control	Straw compost	1,439	...	542	...
Coprinus radians	Straw compost	712	727	362	180
Fusarium sp.	Straw compost	1,131	308	412	130
Mixed fungus flora†	Straw compost	1,260	179	391	151

* Decomposition period 60 days at 28°C.

† Crude culture of a fungus found to be decomposing lignin actively.

The foregoing experiments were repeated using again the alcohol-soluble portion of alkali-lignin from straw and the phenol-lignin prepared from spruce wood. The methoxyl content of the alcohol-soluble and alcohol-insoluble portions of the alkali-lignin was found to be 13.06 per cent and 15.76 per cent, respectively, pointing to certain chemical differences between the two frac-

tions. The decomposition period of the alkali-lignin preparations was 39 days, and that of the phenol-lignin preparations, 49 days (tables 3 and 4). The quantities of lignin decomposed in these experiments were considerably less than those in the previous studies. This may be due to the shorter periods of incubation and possibly to the fact that different sources of lignin were used. Consideration must also be taken of the fact that as a result of decomposition

TABLE 3

*Influence of lignin upon the decomposition of cellulose by fungi**

Milligrams per flask

ORGANISM	CELLULOSE ALONE				CELLULOSE-LIGNIN PREPARATION				
	Cellulose		Nitrate-N left	Lignin left†	Cellulose		Nitrate-N left	Lignin	
	Left	Decomposed			Left	Decomposed		Left	Decomposed
Control	1,337	.....	16.3	0	1,152	...	10.7	495	..
Trichoderma sp	1,120	217	6.0	40	760	392	4.8	489	6
Coprinus radians No. 1	514	823	3.7	83	627	525	1.1	481	14
Coprinus radians No. 2	779	558	3.8	54	423	729	1.7	466	29
Crude fungus culture	303	1,034	0.8	53	757	395	0.1	480	15
Alternaria sp	958	379	5.0	31	718	434	1.3	476	19
Coniophora cerebella	1,206	131	8.7	70	1,042	110	6.8	459	36

* Alkali lignin used.

† Synthesized material.

TABLE 4

Influence of nitrogen source on the decomposition of phenol-lignin in cellulose-lignin preparations

Milligrams per flask

ORGANISM	SOURCE OF NITROGEN	CELLULOSE		LIGNIN	
		Left	Decomposed	Left	Decomposed
Control	$NaNO_3$	1,464	...	497	..
Control	Casein	1,450	...	393	..
Coprinus radians	$NaNO_3$	668	796	489	8
Coprinus radians	Casein	1,363	87	371	22
Fusarium sp	$NaNO_3$	1,164	300	490	7
Fusarium sp	Casein	1,387	77	368	29
Crude fungus culture	$NaNO_3$	931	533	456	41
Crude fungus culture	Casein	1,412	38	383	10

of the cellulose some lignin was synthesized, which amounted to 31–70 mgm. per flask; this would tend to increase the actual quantity of lignin decomposed in the cellulose-lignin preparations.

In order to study the effect of lignin upon the decomposition of cellulose in natural plant material, spruce wood was used. This material is known to be resistant to rapid decomposition by the natural soil population. It was treated in two different ways. The first treatment was carried out with the

purpose of increasing the lignin content and reducing the concentration of readily soluble carbohydrates, especially hemicelluloses and polyuronides. The second treatment had in view the reduction of the lignin content and the increase of the cellulose content of the preparation. As a control for the second experiment, cellulose-lignin preparations with varying concentrations of the two constituents were obtained. Alkali-lignin prepared from straw was used in this experiment. The lignin was dissolved in 4 per cent NaOH solution; the macerated cellulose was soaked in this solution, and the lignin was precipitated by neutralizing the solution with 2 per cent HCl. The excess acid was removed by washing with water. The material was then dried at a low temperature.

For the first experiment, the finely ground wood was extracted thoroughly with benzol-alcohol, with 2 per cent HCl at 100°C., and finally with 4 per cent hot (100°C.) NaOH solution. A portion of the material was removed after

TABLE 5

Decomposition of differently treated preparations of spruce wood by soil microorganisms

NATURE OF TREATMENT	LIGNIN CONTENT	CELLULOSE AND HEMICELLULOSES*	CO_2 LIBERATED	NITROGEN ASSIMILATED
	per cent	*per cent*	*mgm. C*	*mgm.*
Original wood	32.4†	55.4	281.9	37.8
Benzol alcohol	28.7‡	59.9	201.4	31.0
2% HCl§	38.5	52.4	92.0	21.1
4% NaOH‖	43.5	49.7	60.5	20.4

* As determined by hydrolysis with cold 80 per cent H_2SO_4.

† Including fats, waxes, and resins.

‡ Removal of fats, waxes, and resins by treatment.

§ Following the benzol alcohol.

‖ Following the HCl.

each treatment, washed, and dried. Table 5 shows that the benzol-alcohol treatment resulted in the removal of some ligninlike complexes, possibly only the resins, and that the other two treatments brought about the loss of some of the soluble carbohydrates; the alkali also removed some of the lignin. The decomposition of the variously treated wood preparations was carried out as follows: Four 10-gm. portions of the dried materials were placed in 300-cc. long-necked flasks; each flask received 40 cc. of distilled water containing 400 mgm. $NaNO_3$, 200 mgm. K_2HPO_4, 100 mgm. $MgSO_4 \cdot 7H_2O$, and 10 mgm. $FeCl_3$. Two of the flasks received also 1-gm. portions of $CaCO_3$. The flasks were inoculated with a mixed soil infusion and incubated at 28°C. for 47 days. The CO_2 evolved was absorbed in $Ba(OH)_2$ solution. As the quantity of CO_2 produced by the flasks containing $CaCO_3$ and that produced by the flasks to which no carbonate was added, showed comparatively little difference, only the averages of the four flasks are reported. With each successive treatment which led to an increase of lignin content, a marked decrease occurred in the

amount of decomposition of the cellulose in the residual wood. The benzol-alcohol seemed to have removed, in addition to the fats, waxes, and resins, some of the readily decomposable constituents. The lignin and cellulose were determined in this and in the subsequent experment by treatment with 80 per cent sulfuric acid.

In the second experiment, the lignin was gradually removed from the ground wood by means of ClO_2, according to the method of Schmidt (5). For this purpose the wood was first extracted with benzol-alcohol, 4 per cent NaOH in the cold, and hot dilute HCl (2 per cent). The residual material was washed with water and dried. Fresh ClO_2 solution was added, and the mixture was allowed to remain in the dark for 24–48 hours. This was repeated four times, the material being washed thoroughly with water after each successive treatment. Several preparations were thus obtained with decreasing concentrations of lignin and increasing amounts of cellulose. The wood and cellulose-lignin preparations were added to 100-gm. portions of washed sand in amounts

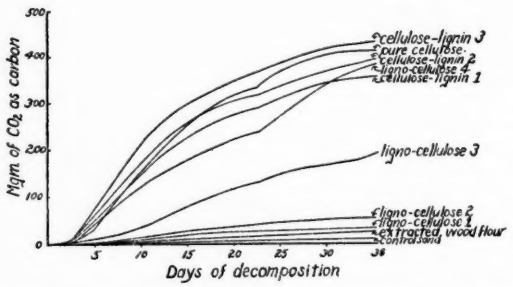

FIG. 1. COURSE OF DECOMPOSITION OF VARIOUS LIGNO-CELLULOSE AND CELLULOSE-LIGNIN COMPLEXES, AS MEASURED BY EVOLUTION OF CARBON DIOXIDE

sufficient to give 2 gm. of cellulose. Water, nutrient salts, and soil infusion were introduced, and the flasks were connected with the respiration apparatus, as in the previous experiment. Incubation took place at 28°C. for 36 days.

The course of evolution of CO_2 is plotted in figure 1, and the chemical analyses are reported in table 6. The results show that in the decomposition of cellulose in cellulose-lignin preparations, the presence of lignin has no injurious effect upon decomposition of cellulose as measured by the evolution of CO_2; in some cases, the effect was favorable, especially during the initial stages of decomposition. In the natural wood, the presence of lignin was found to be decidedly injurious to cellulose decomposition. The removal of the lignin by the ClO_2 treatment up to nearly half of the original concentration had only a slightly favorable effect upon the decomposition of the cellulose by the mixed population. The third treatment, however, resulting in the removal of about 80 per cent of the lignin, led to an increase in the rate of decomposition of the carbohydrates. When the lignin content was reduced to 1.5 per cent, the

decomposition of the cellulose in the wood preparations was about the same as that of pure cellulose.

It is of interest in this connection to direct attention to the fact that Olson, Peterson, and Sherrard have recently found (4) that the inability of anaerobic cellulose-decomposing bacteria to ferment wood is due to the lignin content of the latter. As the lignin is removed, the fermentability of the cellulose is increased. For the purpose of good fermentation, the lignin content must be less than 1 per cent. It was concluded, therefore, that the relation between lignin and carbohydrates in wood is chemical and not physical. No lignin was decomposed under anaerobic conditions.

The results for residual cellulose presented in table 6 do not correspond exactly to the relative rates of decomposition as measured by CO_2 evolution. This is partly because only the cellulose data are reported in the table, the

TABLE 6

Decomposition of cellulose-lignin preparations and ligno-cellulose in wood by soil microorganisms

PREPARATION	BEFORE DECOMPOSITION*		PROPORTION OF ORIGINAL MATERIAL DECOMPOSED		NITROGEN ASSIMILATED
	Cellulose	Lignin	Cellulose	Lignin	
	per cent	*per cent*	*per cent*	*per cent*	*mgm.*
Ground filter paper	93.8	0.5	72.3	....	49.3
Cellulose-lignin No. 1	79.8	16.7	59.5	4.8	60.4
Cellulose-lignin No. 2	67.0	28.0	58.5	15.6	58.7
Cellulose-lignin No. 3	48.2	41.8	54.3	9.0	72.6
Spruce wood†	50.6	44.2	18.0	6.3	2.2
Wood treated with ClO_2 No. 1	54.1	34.5	20.0	9.4	0.1
Wood treated with ClO_2 No. 2	64.1	23.9	19.0	18.1	8.8
Wood treated with ClO_2 No. 3	78.2	8.2	39.0	50.0	25.6
Wood treated with ClO_2 No. 4	91.4	1.5	76.7	57.0	72.7

* Calculations made on ash-free, dry basis; 2 gm. of cellulose used in the different preparations.

† Treated with benzol-alcohol and hot 2 per cent HCl.

hemicelluloses which were left and which were undergoing decomposition not being reported; furthermore, some of the lignin has also undergone a certain amount of decomposition. Cell synthesis by the organisms in the presence and in the absence of lignin was uneven, as illustrated by the figures for the nitrogen assimilation: more nitrogen was again absorbed, for the same amount of cellulose decomposed, in the presence of lignin. This may be due to the fact that lignin binds some of the protein synthesized by the organisms, thus rendering it unavailable as a source of nitrogen for the further growth of the cellulose-decomposing bacteria and fungi; the latter, therefore, have to draw continuously upon the inorganic nitrogen for their synthetic needs. It is also of interest to direct attention to the somewhat larger amounts of lignin decomposed in the wood preparations treated with chlorine dioxide than those decomposed in untreated wood; it is quite possible that some of the lignin has

been so modified by this treatment as to become more readily available to the activities of the organisms.

SUMMARY

A new method is proposed for the study of decomposition of lignin by microorganisms. This method is based upon the solubility of certain forms of lignin in alcohol. The lignin is incorporated upon cellulose fibers, and the cellulose-lignin mixture is used as a source of lignin. Lignin thus prepared was found to undergo a certain amount of decomposition by different organisms, at a much slower rate, however, than the decomposition of cellulose.

Lignin admixed with cellulose had no injurious effect upon the decomposition of the latter. In natural plant materials, however, especially wood, lignin has a marked retarding effect upon cellulose decomposition. When the lignin is removed, the rate of cellulose decomposition increases; however, even the presence of only 8 per cent lignin was sufficient to reduce the rate of decomposition of the cellulose by 50 per cent. When the lignin concentration is reduced to 1.5 per cent, the cellulose becomes as readily subject to decomposition by microorganisms as is pure cellulose.

The depressing effect of lignin upon cellulose decomposition in plant residues cannot, therefore, be considered to be due to the lignin as such, but must be due to the manner of its binding with the cellulose. Whether this binding is chemical or physical in nature, the fact is that it protects the cellulose from rapid attack by saprophytic microorganisms.

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SOME INFLUENCES OF THE DEVELOPMENT OF HIGHER PLANTS UPON THE MICROORGANISMS IN THE SOIL: VI. MICROSCOPIC EXAMINATION OF THE RHIZOSPHERE[1]

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In previous reports (45–49) it has been shown that microorganisms are much more numerous and active on root surfaces than elsewhere in the soil. The numbers of bacteria increased greatly in response to root development; some increase was also noted in the abundance of filamentous fungi and actinomycetes. As was to be expected, the microorganisms responded differently to different plants and to the various stages of growth of any one plant. In addition to the numerous reports discussed previously, there have been several recent publications leading to the conclusion that microbial activity in soils is greatly favored by plant development. Thom and Humfeld (53) observed also that parasitic attack of roots was accompanied by extensive development of saprophytes. Part of the increase about roots of healthy plants might be ascribed to the fact that the plants modified the reaction of the soil about the roots, acid soils becoming less acid and alkaline soils becoming less alkaline. Reuszer (37) found indications of greater biological activity in pasture soil than in bare soil. McKinley (28) noted greater biological activity under maize, sorghum, wheat, and barley than in fallow soil. Truffaut and Lefouin (56) found that the numbers of bacteria increased during growth of wheat and decreased after the plants were harvested.

The influence of plants is particularly striking in soils of semiarid regions where roots penetrate more deeply than in humid regions. Sabinin and Minina found that some sandy soils of arid regions were practically sterile a short distance from the root systems (43). Roots were largely responsible for the relatively large number of bacteria found in these soils at a depth of 5 m. The results of Krassilnikov, Kriss, and Litvinov indicate that the various groups of soil organisms respond differently to plant development (21, 22). Cellulose-decomposing bacteria seemed to take an active part in the decomposition of the roots, but the predominating bacteria were nonspore-formers.

The present studies were undertaken in order to obtain some concrete pictorial evidence of the relations between small roots and root hairs and the soil organisms. It seemed likely that the contact slide method would be useful for this purpose.[2] Rossi and his associates first reported the new technic

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

[2] See preliminary note in *Jour. Bact.* 33: 77 (1937).

by which it was possible to obtain an entirely new conception of the manner in which microorganisms are distributed in the soil (40). Slides or cover slips were pressed against the soil, and the adhering material was stained. Microscopic observation revealed many characteristics of the colony formation of bacteria, filamentous structures of actinomycetes and fungi, and arrangement of the microbial cells with respect to the mineral soil constituents and of certain groups of organisms with respect to others. Slides which had been buried in soil for some time were also examined. This latter method was perfected by Cholodny (2), who first brought it to the attention of most scientists and introduced convincing evidence of its value by photographs clearly showing many typical bacterial colonies and characteristics of the growth of fungi, actinomycetes, and protozoa. The microorganisms and soil particles adhere to the slide surface in a thin film, and, under the microscope, the stained organisms appear in sharp contrast to the unstained inanimate material. The method has been used successfully by Demeter and Mossel (9) to detect changes in the population of field soils in response to fertilizer treatment and plant growth; it has also been used by Conn (8) and by Jensen (13, 14) to follow the changes brought about under laboratory conditions as a result of adding various organic and inorganic substances to soils and of altering the soil reaction, temperature, and moisture content. Jensen (13) made use of the contact slides to determine the relative abundance of Gram-positive, Gram-negative, and acid-fast bacteria in soils. By the same method, Ziemiecka (61–63) noted changes in the nature of the predominating microorganisms in the soil during the course of decomposition of added organic materials. The method enabled Eaton, King, and Hope (10, 16) to demonstrate cases of parasitism of the cotton root-rot fungus in the soil. The contact slide method was also used by Joshi (15) to study the changes in the nitrogen-fixing bacteria in soil, and by Meyer (29) to determine the growth of pure and mixed cultures in sterile soil [see also (59)]. Kriuchkova (23) modified the method by adding films of various agar media to the surfaces of the slides before inserting them in the soil. The organisms which formed colonies could be isolated and studied. Cholodny expressed doubt that this method would be much more useful than the common agar plate (3, 4). All who have used the contact slide procedure agree that it is most helpful in obtaining accurate evidence of the morphological characteristics of the soil population, the aggregation of various organisms, and the influences of environmental factors on changes in abundance, types, and distribution of microorganisms (3, 4, 39).

Most of the evidence concerning localization of microorganisms about roots has been obtained indirectly, by plate counts or by similar procedures. In relatively few instances have microscopic observations been made on roots of plants other than legumes and even in this case only with regard to the nodule bacteria. Zycha (64) planted pea seeds in a mineral agar medium that was inoculated with the pea bacterium, which formed a mantle of cells about

the roots even to the bottom of the tubes. The bacterial development was apparently supported by materials coming from the roots, since no such growth took place about glass rods which were inserted in other tubes of agar. Similar results have been obtained in this laboratory. While some legumes were growing in agar in large glass tubes, a vetch culture became contaminated with a black yeast-like fungus. This organism did not appear to affect plant growth even though it developed abundantly about the roots. It did not make visible growth elsewhere in the agar medium but appeared to be favored in the region of root growth. There is, of course, no assurance that the roots would react similarly in soils.

Rossi obtained fragments of roots and root hairs on his slides prepared from soils, and he reported that there were "no static or numerical relations between the clusters and the absorption apparatus of the plants, but the clusters were concentrated about the roots" (40, p. 65). Since he states that clusters represent resting cells, one would assume that he did not believe that roots appreciably affected microbial development.

Direct microscopic examination of roots disclosed a much more intimate association with microorganisms. Thom and Humfeld (52, 53) reported that the epidermal cells and cortical parenchyma of healthy roots were infested with mold hyphae; bacterial cells were abundant, filling some of the plant cells and being absent or present in small numbers in others. Invasion of the lumen of root hairs by hyphae was not uncommon; ". . . each rootlet and each root hair is fringed with microorganisms. . . . All around these complex branching systems swarms of microorganisms line every pore of the soil" (51, p. 57–58).

The contact slide method was used by Hulpoi (11) in much the same manner as that in the experiments to be presented. He placed cover glasses in soil in which plants were grown. The plant roots passed over the glasses, and some became attached. After these preparations were stained, the influence of the roots on microbial development could be detected by microscopic observation. Photomicrographs illustrated localization of rod-shaped bacteria on or in root hairs of oats and lupines; in general, the bacterial cells were scattered, but in other cases they developed in dense aggregates on the root hairs. The evidence emphasizes the condition described by Thom.

EXPERIMENTAL METHODS

The contact slide method was used in much the same way as that described by Cholodny (2). Microscope slides were inserted in soil in the field, plants were grown in the soil, and the slides were periodically removed and examined. The soil is classified as Sassafras loam and varied in reaction between pH 6.0 and 6.8 during the season. Slides twice the usual size (52 by 77 mm.) were placed in the soil in a vertical position. Seeds or seedlings were then planted 1 to 3 inches above the slides. Seeds of the following plants were used: mangel beet, barley, maize, rape, vetch, and soybean. Pepper and tomato

seedlings were also planted. A portion of the field was kept free from all vegetation, and slides from this region served as controls to indicate the nature of the soil population in the absence of root development.

It was anticipated that as the plants developed, some of the roots would pass over the surfaces of the slides and that some portion would adhere to the surfaces. Roots did not appear, however, on all the slides. After the slides were taken from the soil one side of the slide was cleaned, the larger soil particles were removed from the other side, and the slides were dried and then stained with phenolic rose bengal (8) for 10 minutes. During the staining period the slides were kept warm over boiling water. After being washed and dried, the preparations were examined under the microscope, a 2-mm. apochromatic objective (n.a. 1.30) and 12.5 x compensating oculars being used. The immersion oil was added directly to the preparation without use of a cover glass. The entire surface of each slide was carefully explored, the abundance of microbial cells, characteristic formations, and particularly the relationships of the organisms to roots and organic detritus being noted. The slides were taken from the soil during the summer of 1934 and have been examined at various intervals during the last 3 years. Representative formations were photographed. At first a Reichert camera with which photomicrographs could be obtained only up to magnifications of 650x was used. Most of the photomicrographs, however, were made with a Bausch and Lomb Type H camera, a magnification of 1200x being obtained on the film. None of the illustrations in this paper have been retouched, and all are presented as obtained from contact prints.

NATURE OF THE SOIL POPULATION

On the slides from fallow soil or on portions of the other slides where roots had apparently exerted no influence somewhat limited microbial substance occurred, but diverse types of organisms were encountered. The organisms were more or less well distributed as colonies, the individuals and their arrangement suggesting orderliness.

Bacteria

Many of the bacteria occurred as isolated cells; some were coccoid, and others were thin or thick rods or spindle-shaped cells. There was no lack of scattered isolated cells. Much more prominent were the small aggregates of bacterial cells. The predominating forms were small coccoid or spherical cells, although a few larger ones were seen. Some of the larger cells, most of which were deeply stained, formed tetrads or packets; many of the pairs of cells were bean-shaped (fig. 1). Most of the cells were in compact aggregates and seemed to be imbedded in material which retained some of the stain. Many of the aggregates were associated with soil material. Colonies of these organisms were considered by Winogradsky to be typical representatives of his soils (60). Photographs by Romell (38, pl. 1A), by Demeter and Mossel

(9, fig. 2b), and by Koffman (17, fig. 21) show cells of somewhat similar appearance. Although many such organisms were seen on virtually all the slides, they were not the most common forms. Larger spherical cells in packets imbedded in lightly staining microbial substance (fig. 2) also occurred. They are considerably larger than most of the bacteria that were encountered, and it is possible that they are not bacteria.

The bacteria seen in greatest abundance were small, nearly spherical cells, commonly encountered in thin spreading aggregates or in denser groups rather variable in size; although some of the colonies were larger than the one shown in figure 3, most of them were small. Compact cyst-like masses, like the one in figure 4, would probably be typical of the clusters described by Rossi as inactive organisms (40).

Some idea of the abundance of these colonies of small coccoid cells can be gathered from the fact that about 300 such colonies were recorded on a single slide from the fallow soil even though no attempt was made to record all the colonies. These small coccoid cells in small and large aggregates were generally located in the midst of some mineral or organic material. They are not readily distinguished in photographs, since relatively few cells are in focus at any one time and the soil material obscures the details. As with all of the preparations, cells which are clearly distinguished by the ruby-red color lose much of their striking appearance in the black and white photographs.

An explanation for the predominance of cells of coccoid shape was advanced by Conn (7), who believed that the bacteria assume the nearly spherical shape under conditions of low nutrient level in the soil environment and that they become longer rods at times when they have access to readily available food material. The relatively scant microbial development in fallow soil and the arrangement of the cells further emphasize the fact that the fallow soil is poor in microbial food. A great change occurs where plants are growing and root parts become attacked, as will be discussed later.

Other cells, shown in figure 5, appeared to be cocci in tetrad formation but were actually rod-shaped cells having deeply stained ends; few organisms of this type were noticed. Larger rod-shaped bacteria were generally associated with decomposing bits of organic matter. Many thin, spreading, veil-like films of bacilli were encountered, however, without any evidence of organic materials undergoing decomposition nearby (fig. 6). Some of these colonies were very large and spread over an area equal to several of the microscope fields. A similar formation is shown by Cholodny (2, fig. 16). A few loose colonies were seen composed of fairly long cells tapering to points at the ends (fig. 7).

Cells like those shown in figure 1 suggest the appearance of Azotobacter, but pairs of spherical cells shown in figure 8 and particularly the colony of fairly large unevenly stained cells in figure 9 [see (2, fig. 18)] are more typical of this organism. Although such cells have been seen on many of the slides, they were not typically associated with any organic substances, nor

were they abundant enough to suggest that they were particularly active; several colonies of these cells were seen about rootlets.

Other colony types and bacterial shapes were encountered, but in such small numbers as to suggest that relatively few of them were present in the soil under consideration. Few spore-forming rods were seen (fig. 11); some other large rods, which may have been spore-formers, appeared. Two groups of long slender cells suggestive of the cellulose-decomposing bacteria of the genus Cytophaga were noted in the vicinity of root detritus on slides from maize (fig. 12). The cells stained well in the center and very lightly at the ends, which were pointed. Two groups of organisms of very unusual shape were seen on one of the slides from vetch. One of these colonies was composed of large, uniformly stained, vibrio-shaped cells (about 3μ long) pointed at the ends (fig. 10). Although they were somewhat larger than the common vibrios it is probable that they were bacteria. The other more unusual cells shown in figure 13 were lightly stained tubular rods more than 20μ long, having a deeply stained portion near one end which looked like a fairly large bacillus (about 2μ long). These cells are unlike any bacterial forms previously seen. On a slide from beets, a colony of fairly long rod-shaped cells occurred in palisade formation, much like those shown by Cholodny (2, fig. 16). A few typical sarcina packets of small cocci were noted.

It should not be concluded that the slides were covered with organisms, for the majority of slides had many more fields with no apparent microbial cells than fields showing microbial development.

Actinomycetes

Actinomycetes appeared in abundance on all slides, both in conidial forms and as unfragmented filaments, the conida being considerably more common. This agrees with Conn's observation made in 1918 that the conidia are so much more numerous than the filaments that undoubtedly virtually all the colonies of actinomycetes on the plates arise from the spores (6). It fails to support the contention of Lutman, Livingston, and Schmidt (27) that the organisms exist mainly in the soil as bits of mycelium with fewer spores. The differences of opinion are no doubt due to the difficulty in distinguishing conidia from bacterial cells in disturbed soil. It is readily understood from the large number of conidia and actinomycete filaments why such a large portion of the colonies on agar plates are formed by these organisms. As emphasized by others, the method more accurately portrays the true morphological characteristics of the actinomycetes in their relation to the soil environment than does any other method. The cells stain exceedingly well and disclose structures which are not readily detected by other means. The actinomycetes were generally stained better than any of the other organisms, but even with such fine preparations it was impossible to tell whether certain rod-shaped cells were bacteria or conidia of actinomycetes.

Many of the conidia were scattered and occurred in small aggregates of a

few cells or in short chains. Others, however, were encountered in large groups composed of many conidia including complete and broken spirals and fine nonfragmented filaments. Some of the aggregates of fragmented filaments are shown in figures 14, 15, and 16. In many instances the cell material was very profuse, covering an area of many fields of the microscope, thus indicating that there had been considerable decomposition of organic material. In figures 15 and 16, shadows of fine filaments appear in the background; it seems likely that they represent the vegetative mycelium of the actinomycetes, which is commonly finer than the fragmented aerial mycelium. Generally the actinomycetes were free of bacterial associates on the slides from the fallow soil and on those portions of the other slides where roots had not penetrated. The illustrations of bacterial colonies further emphasize the fact that very many, if not most, of the bacterial aggregates are also composed of single-cell types where readily decomposable organic matter is not present. Some associations of bacteria and actinomycetes were encountered, however, and occasionally both organisms developed profusely together. A very few actinomycete filaments were studded with bacterial cells, suggesting bacterial decomposition of the filaments.

In addition to the scattered conida, many branched mycelia bearing spring-like coils of fragmented filaments were encountered. Some of these formations are shown in figures 17, 18, and 21 (see also figure 57). Some of these coils were loose and open, and others were pressed closely together. Many of the spirals were broken apart, only remnants of the original formations being apparent. The coiled conidial filaments were coarser than the filaments from which they originated, and fragmentation was more readily seen in the coils. Other sporulating spirals were seen, the coils of which were in one plane, appearing like concentric circles of fragmented conidia. In figures 17 and 18 more than one type of fragmentation is apparent; most of the conidia are relatively close together, but others are well separated and appear to have constrictions between them. In figure 16 the conidia seem to be retained in a thin sheath; this is the type of spore connection most commonly observed. The spores pictured in figure 19 show no such connecting sheath but are held together by a thin strand. Whether the differences are typical for different species of actinomycetes or are indicative of different stages of maturity of the spores or whether they are brought about during the staining of the slides is not known.

The loose open growth of the colonies of the actinomycetes, even though spreading over a large number of microscope fields in some cases, re-emphasizes the fact that the soil medium is comparatively deficient in readily available nutrients.

An unusual type of formation was encountered in several places on one of the slides from the vetch soil (fig. 20). From a group of deeply stained small coccoid bodies, fine branched filaments radiated. It seems most likely that these were germinated spores of an actinomycete showing the early stage of

mycelial development. Many ropelike coils of actinomycete filaments like those shown by Demeter and Mossel (9, fig. 11a) and by Jensen (13, fig. 9) were also seen.

Filamentous fungi

Fungus filaments occurred in considerable abundance even on the slides from the fallow soil; many extended in all directions and were visible without the aid of the microscope. They were generally somewhat lightly stained, ribbonlike threads, but many short or long pieces of large, unstained, brown, septate mycelium were also seen. A great variety of spores occurred which were prominent by reason of their large size in comparison to the cells of the bacteria and actinomycetes. Among those most frequently observed were somewhat distorted nearly spherical spores about 2μ in diameter, belonging probably to the Fungi Imperfecti; a few scattered spores are shown in figures 63 and 64. Fusarium spores shown in figure 22 appeared singly and in large groups. Spores like those in figure 29 were seen more or less frequently on all of the slides. They were large cells (more than 20μ in length) containing four deeply stained bodies within the almost clear elongated envelope; a fifth dense body at one end protruded and undoubtedly was the point of attachment to the mycelium. They were probably spores of Helminthosporium or of a closely related genus. Dark brown unstained multicellular spores characteristic of Alternaria and related fungi were seen, and also a few dark brown, two-celled, egg-shaped spores like those in figure 30. The spores shown in figure 23 were virtually the only cells which did not remain in place on the slides covered with immersion oil. They floated in the oil and became dispersed. They were flattened circular spores having numerous striations which gave them a cogwheel appearance in one position. They were hollow on one side, and the general shape closely resembled that of the cap of an acorn. The unusual cells shown in figure 24 are also believed to be fungus spores. They were shaped like six-pointed stars with a deeply staining central body. Many other spores were seen, including large oval cells, elongated rod-shaped cells, spheres, and irregularly shaped cells which may have been distorted by the staining procedure; some of these stained deeply, and others were unstained brown spores.

Although almost all the spores were scattered, not many being attached to the sporangia, a few sporulating bodies were seen. The whorls of sterigmata in figure 25 characterize this as a Penicillium. Figure 26 shows a strange sporulating body. Several such structures arose from the same mycelial strand at distances of about 75μ apart. A bicellular or multicellular swelling occurred part way up the sporulating hypha; above this was a smaller swelling, and above this, a large spindle-shaped body that looked like a Fusarium spore. A filament bearing relatively large, single pear-shaped spores on each of the short hyphae is shown in figure 27. This organism is probably closely related to the genus Sporotrichum. A sporangium typical of the Mucoraceae was

also seen. The rectangular vacuolated cells in figure 28 are probably related to Monilia or Oidium. This illustration also includes a large nearly spherical fungus spore with a small pointed projection.

As has been frequently emphasized, the contact slide method reveals an abundance of fungus filaments in the soil, leaving no doubt as to the vegetative development of fungi in soil even in the absence of appreciable amounts of readily decomposable organic matter.

Algae

The most frequently distinguished algal cells were diatoms. Bristol-Roach stated (1) that although diatoms occur rather commonly in the soil, there is a great preponderance of green algae, mainly of the unicellular forms, in temperate zones. Very few algae other than diatoms were detected during these observations, probably because the large soft cells of the green and blue-green algae became so deformed by the desiccation and staining procedure that their identity was not apparent. Siliceous skeletons of the diatoms, free from all protoplasm, were common. Probably all of the diatoms which were seen belong to the group of Pennatae. Only one of the forms, that shown in figure 31, was characteristic enough in appearance to permit identification; it is probably *Hantzschia amphioxys* which has been found frequently in the soil (58). The majority of the cells were shaped like those illustrated in figures 31, 32, and 33. Numerous striations and pits could be seen. The striations sometimes extended over the entire surface of the skeletons as with the forms in figures 31 and 32. On other cells, such as those shown in figures 33 and 35, the striations seemed to be confined to the ends and ran diagonally toward the center of the skeleton. There were also various other forms, including the one in figure 34, spindle-shaped cells resembling Navicula, and cells considerably longer and more slender than any of those illustrated. Occasionally they were present in pairs or in small aggregates, but generally they were solitary. Shrunken protoplasmic contents can be seen in the forms pictured in figures 32, 34, and 35. Illustrations of diatoms obtained from soil preparations are shown by Wang and Chia (59), Koffman (18), and Demeter and Mossel (9). Some of their specimens resemble those mentioned in the foregoing description.

One chain of rather large nearly spherical cells was noted (fig. 36). Each cell contained several deeply stained particles located against the cell membrane. Their appearance suggests blue-green algae such as Nostoc.

Protozoa

No material was seen which was identified definitely as a protozoan. Undoubtedly many of the deeply stained, large, irregular bodies which were observed were trophic stages of protozoa; some of the dense spherical and similarly shaped cells were probably cysts.

Invertebrates

On each of the slides were skeletal remnants of some small animals, those most commonly seen being thin striated scales with an attachment point at one end, and thin tapering spiny setae (fig. 37). These materials were generally scattered but occurred in many places in large masses, each of which looked like most of the chitinous covering of a tiny animal; frequently claws were detected at one end of an elongated group of setae. The scales varied considerably in size. Some of the setae were smooth, and some were covered with spines; some were hollow, and others appeared to be solid.

Occasionally some filaments of fungi or actinomycetes appeared about these materials, but more commonly there was no evidence of microbial development about these resistant organic substances. There was more apparent microbial attack of a small animal shown in figure 38. Legs and body parts were still well preserved, but attached to them and radiating in all directions over many fields of the microscope was a fungus bearing single spherical spores on short slender hyphae. The fungus is probably a member of the Dematiaceae. The fungus development illustrates the localization of microorganisms in regions where food material is available. In such cases, however, the fungus may be responsible for the development of other organisms at some distance from the location of the animal, since its mycelium, which spreads for some distance, eventually would be attacked by bacteria or other microorganisms.

Nematodes were seen in only three places. A portion of one showing the head and the upper part of the body can be observed in figure 66; this nematode was in contact with a small root. The forms encountered were from 200 to 400μ in length.

Localization of microorganisms about organic detritus

The arrangement of the microorganisms with regard to other soil materials on the slides has repeatedly emphasized the fact that microbial development is extremely localized. Cells grow only where there is food, and large accumulations of cells are to be found only where there has been an abundant supply of such food. Humfeld and Smith (12) found that although bacteria were extremely numerous throughout a mass of green manure undergoing decomposition in the soil, the effects of the manure were very local, and the abundance of bacteria was not greatly modified a short distance away. Similar localization about organic materials was observed by Krassilnikov (20). Localization of a fungus about a small animal has been mentioned. Aggregates of bacteria and actinomycetes were very frequently encountered about bits of organic matter. Figures 39 and 40 are representative of this condition, showing considerable numbers of small rod-shaped and coccoid bacterial cells about and on the organic material. An extremely extensive dense colony of longer and larger bacilli is illustrated in part in figure 41. This

colony occurred on a slide from the vetch soil. It spread over 15 to 20 fields of the microscope, disclosing scattered bacterial cells and occasionally some septate fungus mycelium, shown in the picture, and actinomycete filaments. This was the largest mass of bacterial cells encountered at any one place on the slides.

The predominating bacteria in the colonies about organic matter were small coccoid cells; longer rods such as those shown in figure 41 were less common, and spore-forming rods were very rare. Groups of fungus spores were detected in some of these regions of active decomposition.

Localization of microorganisms about fungus filaments

Even more striking than the localization of microorganisms about organic detritus was the very common and extensive development of bacteria about fungus filaments (2, 8, 9, 13, 61, 62). Mycelium was abundant on most of the slides, and a considerable number of bacteria were in close contact with much of it. Such bacterial-fungus associations were not seen with the brown mycelium. The bacterial cells were most commonly scattered along the hyphae or in small aggregates (fig. 42). Some of the filaments were studded with small uniformly sized colonies (fig. 45). Many of the bacterial aggregates were large and dense (fig. 43), being composed of hundreds of tiny, lightly stained, coccoid cells difficult to resolve in photographs (fig. 44, 51). These large groups were particularly numerous on slides from the soils in which rape and vetch were growing. The small coccoid bacteria were by far the most common fungus associates, although occasional larger rod-shaped cells like those in figure 46 were seen. There is little doubt that these formations represent stages in the destruction of the filaments by the bacteria. This is emphasized by the fact that the filaments took very little stain, indicating the absence of appreciable amounts of protoplasm. One may be justified, however, in questioning how masses of bacterial cells, which seem to have considerably greater volume than the fungus filaments in the immediate vicinity, could have grown from the protoplasm of these filaments alone. It seems necessary to assume that they grew in part from products of the fungus growth; at least some of the latter must have been transported from other portions of the mycelium.

Fungus mycelium thus seems to be very susceptible to bacterial attack and probably does not generally persist for long in the soil, having at least shorter existence than many of the bacteria and actinomycetes. Certain environmental conditions may lead to rapid development of fungus mycelium which will soon be destroyed following exhaustion of the limited food supply or a change in the environmental conditions. This would explain in part the reason why, in response to certain soil treatments, comparatively little of the anticipated increase in abundance of fungi is detected by the plate method. Jensen (14) concluded that the contact slides were more satisfactory than plate counts for determining the density of fungus mycelium in the soil.

Localization of microorganisms about roots

The slides from soils supporting plant growth showed not only all the qualitative characteristics apparent on the slides from the fallow soil but also numerous additional ones. Quantitatively the population was much more dense even where there was no evidence of root material in the immediate vicinity on the slide.

Slides free from roots were characterized by well-defined and almost neat development and regular scattering of various colonies and occasional cells. In the presence of root material the picture was greatly altered. There were many irregular aggregates of great numbers of various organisms indicating rapid and extensive cell development, typical of what Thom (50) refers to as the "explosive" type of growth. Here may be found bacteria, actinomycetes, and fungi developing together in a confused arrangement. The condition may persist for a considerable distance from the location of the apparent root material, although most of the cells are confined to the roots. The greater the apparent degeneration of the root parts, the greater is the variety of the microbial invaders. Considered as a whole, the slides from planted soil supported much more profuse development of microorganisms than did slides from fallow soil.

The organisms are more readily illustrated about root hairs than in contact with larger roots, since the root hairs are generally lightly stained and reveal the more deeply stained microbial cells. Some typical root hair formations are shown in figures 47–50, 52–56, 58 and 60, some of which are similar to Hulpoi's illustrations (11). The ribbon-like root hairs support occasional fairly large rods, but more commonly very tiny coccoid cells are found, many of which are in chains either within the root hairs (figs. 47, 48, 52) or spread over the surfaces and radiating from them (figs. 53, 54). Most of the hundreds of root hairs attached to the small vetch root from which figures 48 and 54 were obtained were permeated with chains of these cells. These threads of tiny cells resemble actinomycetes in many respects, particularly since some of them appear to be branched (figs. 48, 61, 62). The filaments are smaller, however, than most of the actinomycete conidia. Furthermore, previous results (48) indicated that actinomycetes are not very much more abundant about roots than in soil free from roots. If such cells as these are actinomycetes, and if they invade roots as generally as these microscopic studies indicate, much greater numbers of actinomycete colonies should have been obtained on the plates from the root samples. It seems most likely that these are chains of small bacterial cells.

On some root hairs the bacterial cells were scattered and occurred in relatively small numbers. Occasional small colonies (fig. 58) were seen. Many chains of bacterial cells developed in close contact with the exterior of the root hairs, giving the edges a beaded appearance (figs. 47, 53, 58). In figure 49 the bacteria are shown in the form of a mantle about the tip of a root hair.

Large numbers of bacterial cells were localized about a root and root hairs shown in figures 55 and 56, some of the cells being scattered on the root surfaces and some forming colonies near the roots. In figure 59 groups of bacteria are shown about a clear zone which was probably occupied by a root hair or small root which became dislodged during the preparation of the slide.

It is not possible to state definitely whether or not the root hairs were in a vigorous condition at the time the photographs were made. In many regions of microbial development it was difficult to determine whether the material undergoing decomposition consisted of dead roots, sloughed-off cells, dead root hairs, or root excretions. Many of the root hairs, however, were followed from their point of attachment to the small roots throughout their entire length, and they appeared to be intact except for the microbial associates. Many of them showed deeply stained portions indicating the presence of considerable amounts of protoplasmic material (figs. 47, 48, 49, 54, 58, 60). Microorganisms were not detected on all the root hairs; however, their common occurrence seems to justify the conclusion that bacteria develop in abundance upon apparently vigorous root hairs. The attack of the latter by microorganisms is so rapid that it is unlikely that appreciable residues persist long after death of these root parts. Large numbers of bacteria were also seen on small roots.

Microbial development was not confined to the bacteria. Relatively large, branched, septate mycelium is quite evident in figure 48. Fungus mycelium was in evidence about many roots but not in such great abundance as were the bacterial cells. Well-defined development of actinomycetes was more apparent than that of fungus mycelium. Short conidial branches, unfragmented filaments, and aggregates of conidia were seen about the root hairs (fig. 60) but in small amounts compared to the cell material of the bacteria. The actinomycete shown in figure 57 appears to have grown from a small root. The extensive mycelium which spread over many fields of the microscope indicates that considerable decomposition of organic matter had occurred. The coiled sporulating hyphae clearly illustrate the characteristic morphology of the soil actinomycetes. The fragmented conidia shown upon the root hair in figure 50 might easily be confused with bacterial cells.

Considerable evidence of more advanced microbial attack of root hairs was discovered, particularly on slides which had remained in the soil for several weeks, some of the preparations appearing much the same as the pictures by Hulpoi (11, figs. 4 and 5). Figures 61, 62, 63, 64, and 66 are typical of this decomposition of roots. The root hairs in figures 61, 63, and 64 are shadowy in outline and are spotted with bacterial cells, actinomycete conidia, and fungus spores. Filaments of actinomycetes form connecting networks between many of the root hairs. About some of the microbial aggregates there were residual root parts, and in some places only masses of bacterial cells and filaments of actinomycetes and fungi remained where the roots had

been located. Bacteria and actinomycete filaments can be seen in figure 66 in the vicinity of a larger root; the dark projection with a lighter end is the head part of a nematode, the sinuous body of which was in close contact with the root. A portion of a relatively large root is shown in figure 62. Much of the organic material had been decomposed, and in its place appeared chains of tiny coccoid cells forming a profuse network. They have much the same appearance as the chains of cells commonly encountered in the root hairs (figs. 47, 48, 50, 52, 53, 54). Great numbers of larger rod-shaped bacterial cells were also present in other regions of this decomposing root, and masses of bacterial cells as well as filaments of actinomycetes and fungi radiated from it in various directions. In figure 65 small coccoid cells appeared in a mass, projecting from, as well as at a short distance from, a large root where a small root may have been decomposed.

Some roots of vetch were completely covered with masses of bacterial cells. They stained so deeply that it was difficult to obtain a good reproduction of the individual cells which composed the aggregates (figs. 67, 68). There were even swellings upon the roots where the organisms had accumulated in large numbers. Actinomycete filaments radiated from these roots; some fragmentation is evident, and a well developed spiral can be seen in figure 68. There is little doubt that these roots were almost completely decomposed, since only short portions could be found on the slides and very little root material remained, the root outline being preserved principally by the microbial cells.

Specific influences of the various plants

There was very little evidence of differences in the response of the microorganisms to different plants. Although the extent of microbial development was not the same on all slides, it seemed to be determined more by the abundance of root residues on the slides than by the type of plant growing in the soil. The slides from vetch consistently had large numbers of organisms, but one of the slides from the beet soil had the most profuse microbial development of all the slides examined. Only vetch roots were found completely covered with bacterial cells in the manner shown in figures 67 and 68. Fungus filaments were particularly abundant on some of the slides from the rape soil. There is little justification, however, for concluding that such conditions are specific for these plants. More closely controlled conditions and more numerous observations are needed to obtain information as to the specific organisms which are particularly favored by any one plant and as to the characteristic root formations of any plant.

Likewise there were no striking differences in the appearance of the slides which were removed from the soil at various periods after the planting date. No attempt was made to determine the conditions about roots of very young plants. The first slide was taken from the soil 23 days after planting (June 28), and the slide which remained in the soil for the longest period was re-

moved 128 days from the time of planting. After the slides had remained in the soil for several weeks, they showed more root material and associated organisms and considerably more roots in somewhat advanced stages of decomposition than did the slides which were first removed. Otherwise, and except for differences in the relative abundance of microbial cells, the slides had much the same microscopic appearance, irrespective of the age of the plants when the slides were removed from the soil. Some differences undoubtedly could have been obtained by varying the experimental procedure.

DISCUSSION

Although there is considerable microbial development in contact with intact roots, a large part of the root hairs and surface of larger roots appeared to be free from microorganisms. By far the greatest microbial development seems to be at the expense of dead root parts. Most of the organisms are unquestionably saprophytes concerned in the decomposition of root residues, but some of the organisms may be of more significance in the development of plant roots. Thornton (54, 55) obtained evidence that some substance is excreted from the roots of legumes which stimulates infection of the root hairs by the nodule bacteria. Ludwig and Allison (26) observed that maize growing in association with the legumes also favored legume nodulation, presumably through some organic materials originating from the maize roots. Virtanen has found that large amounts of organic nitrogenous materials are liberated from the roots of inoculated legumes [(57) see also (31)]. In a recent review, Loehwing recorded considerable evidence of the excretion of both organic and inorganic materials from roots (25). The ability of certain legumes and other plants to absorb phosphorus from relatively unavailable materials (25, 35, 36) has been ascribed to the excretion of organic acids from the plant roots (30, 42, 43). It thus seems likely that the development of microorganisms on roots might be brought about in part by organic excretions which the organisms use as food material.

Sherman and Hodge (44) found that at least some plants contain material having bactericidal properties. If this is a condition common to all plants, microbial development within intact tissues should be rare except in cases of parasitism or such special cases as nodule development by the legume bacteria (48).

Other factors which are concerned with the development of microorganisms about and within roots have been discussed previously (41, 45, 48).

The microorganisms which grow in the rhizosphere also affect growth of higher plants. In addition to the effects of the common products of microbial development (45, 48), certain organic substances (hormones, vitamins, plant stimulants) may be produced by microorganisms and affect the rate of plant maturity, cell growth, and root formation (24). Niethammer found that some of the common soil fungi favored germination of seeds and seedling development in sterilized soil and in agar medium (32).

It was not surprising that cells resembling Azotobacter were encountered somewhat infrequently and in very small numbers in comparison with the total number of bacteria. Although it is logical to suppose that the rhizosphere is a favorable region for development of the nitrogen-fixing bacteria (48), and even though Azotobacter has been recovered from plant roots (33, 34), in no case has it been demonstrated that these organisms are present in sufficient abundance to be of particular significance in the growth of higher plants. The claim of some Russian investigators (Kostytschew, Sheloumova, and others) that plant growth is improved by inoculating the soil with Azotobacter is not completely convincing (24). Krassilnikov concluded that the rhizosphere of maize and wheat does not favor the development of Azotobacter but that such bacteria as *B. denitrificans* and *B. fluorescens* grow well about roots of these plants (19). Since he used solution cultures, his results may not be typical of what occurs in the soil.

Although the amount of bacterial cell substance appeared to be greater than the amount of any other microbial material, conidia and filaments of actinomycetes were very numerous and at times more abundant than the bacterial cells. Conn reported some years ago that actinomycetes are particularly numerous in sod land and are active in the decomposition of grass roots (5). The present results indicate that actinomycetes are to be found in abundance about root materials of many plants and are commonly encountered, even though less abundantly, throughout the soil even in the absence of root development. Ziemiecka (61, 62) classified actinomycetes as secondary organisms which follow either bacteria or fungi in the decomposition of organic materials. No exact information concerning this point has been derived from the root studies, but, since cells of actinomycetes are commonly encountered even where there is evidence of decomposition in the absence of other organisms, it is unlikely that actinomycetes are active solely in advanced stages of decomposition.

It is not possible to make an accurate estimation of the relative abundance of fungus material; although the filaments and spores are much less frequently encountered than are the cells of bacteria and actinomycetes, the amount of cell material represented by a single fungal hypha would be as great as that contained in numerous bacterial cells. It is quite likely that in many places the fungus material exceeded the amount of substance in the cells of both the bacteria and actinomycetes. As has frequently been noted, numbers of organisms as estimated by plate counts give an erroneous impression of the significance of the various representatives of the soil population; this is particularly true with the filamentous fungi. From the fact that bacteria were commonly localized about fungal hyphae, it can be concluded that fungi were responsible for a considerable portion of the increase in numbers of bacteria in the rhizosphere.

It is still uncertain why very little influence of plants on abundance of fungi was detected by means of the plating method whereas the slide preparations

show a pronounced increase in fungus development under growing plants. It may be that the bacterial cells are viable for a longer period than is the fungus mycelium, or that the fungi produce relatively few spores, or that the media which were used for plating were not suitable for cultivation of the predominating soil fungi. The brief existence of fungal hyphae in the soil and the inadequacy of the plate method for determining the amount of mycelium are probably responsible for the lack of agreement in the results obtained by the two methods.

The illustrations included in this paper reproduce a few of the great number of varied formations which characterize the development of microorganisms in the soil. They show some of the most typical formations, those which were particularly obvious, and those which appeared to demonstrate interesting relationships between the microorganisms and the soil materials. Although they convey but a small portion of the complete picture of the arrangement of the microbial cells about the soil particles and the associations of various organisms, still they give some suggestion of the actual manner in which these microorganisms exist in their natural habitat.

The contact slide method has been extremely useful as a means of obtaining this evidence, particularly that concerned with the localization of the microorganisms about roots. The method provides a means of obtaining a more accurate conception of the development of microorganisms in response to root growth, the types of organisms affected, the localization of the individuals and groups, and the sequence of morphological types. However, it has certain limitations (3, 4, 13). It is unfortunate that there is no possibility of cultivating the organisms which are seen on the slides. Nothing can be ascertained concerning the physiology of the microorganisms which are encountered, and it is practically impossible to recognize specific bacteria on the basis of morphology alone. Furthermore, it is necessary to speculate as to the time the microorganisms developed upon the slide. When examining slides which have been in the soil for several weeks, it is not possible to state precisely when or under what conditions these organisms appeared. These obvious limitations, however, do not appreciably affect the value of the method for morphological studies. Certainly the present studies have made it possible to visualize more clearly some of the details of the root-microorganism associations which were known to exist from the results obtained by the plate method. The results emphasize the exactness with which Thom described the root conditions responsible for microbial development (50, p. 161):

> This bacterial distribution is directly dependent upon continual production of new epidermal cells, parenchymatic cells and root hairs at the growing root tip. The root-cap consists of soft walled parenchymatic cells which are constantly renewed from within and continuously dying and decomposing on the outside with remains of the outer cells crushed into the surrounding soil. Similarly the epidermal and cortical cells and the root hairs of the growing root itself, function actively only for a short time, after which they "slough off." This is readily seen under the microscope to mean that they are rotted by moulds and by countless numbers of bacteria.

SUMMARY

The buried slide technic was used to determine the nature of the development of microorganisms about roots of growing plants. The method proved to be useful for demonstrating some of the colony formations and growth characteristics of various soil microorganisms. Some of the organisms which developed in response to root growth and the types of microbial formations on root hairs were readily observed.

As had also been determined by the plate method, microbial development was found to be much more extensive about roots than elsewhere in the soil. The effect of roots is rather local, a large portion of the organisms developing in close contact with the roots and root hairs. The mycelium of the filamentous fungi may spread for some distance from the organic matter which is being decomposed. Small coccoid bacteria commonly appear in abundance on the fungus mycelium. Bacteria were found as scattered cells and in small aggregates about root hairs, conidial fragments and filaments of actinomycetes were recognized, and branched filaments and scattered spores of filamentous fungi were seen.

Microbial development was most extensive where dead root material was decomposing. In such regions rather large masses of cell material and many different organisms occurred in a confused arrangement, bacteria and actinomycetes predominating. The microorganisms were not confined to dead plant substance but occurred in considerable abundance about, upon, and within roots and root hairs that seemed to be in a vigorous condition.

The bacteria occurring in greatest abundance about the root hairs, fungus filaments, and decomposing organic matter were small, coccoid, lightly staining cells. Longer rods were detected, but spore-formers were seldom encountered.

No striking differences were noted in the types of microorganisms associated with different plants. The most apparent change in the population as the plants became older was the increase in abundance of organisms and the greater amount of decomposing root material.

In addition to the microorganism-root associations, many characteristic types of bacterial cells and colony types were seen, including small coccoid cells, Azotobacterlike cells, rods, fusiform organisms, and a large vibrio. Actinomycetes were represented mostly by fragmented conidia; springlike coils of sporulating filaments and branched vegetative mycelium were frequently encountered. Fungus mycelium was abundant, various types of fungus spores were seen, and sporangia of a few different fungi were noted. There were several types of diatoms, but no cells typical of protozoa were recognized. Chitinous remains of many small invertebrates were found.

Various microbial cells were encountered on the slides from the fallow soil, but the cells were less numerous and more uniformly distributed in colonies and in small scattered aggregates than where roots had penetrated.

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PLATE 1

FIG. 1. Small colony of relatively large bean-shaped bacterial cells. From rape; slide in soil for 126 days. 1200 ×

FIG. 2. Packet of large, nearly spherical bacterial (?) cells imbedded in capsular material. From vetch; slide in soil for 128 days. 1200 ×

FIG. 3. Very small coccoid bacterial cells in a loose colony. From maize; slide in soil for 40 days. 1200 ×

FIG. 4. Oval, compact, cystlike bacterial colony about soil material. From maize; slide in soil for 40 days. 1200 ×

FIG. 5. Loose colony of terminally staining bacilli about soil material. From vetch; slide in soil for 71 days. 1200 ×

FIG. 6. Large, spreading veillike colony of lightly staining bacilli. From vetch; slide in soil for 53 days. 1200 ×

FIG. 7. Loose colony of fairly long, spindle-shaped bacterial cells. From maize; slide in soil for 40 days. 1200 ×

FIG. 8. Spreading colony of fairly large spherical cells, commonly in pairs (Azotobacter?). From fallow soil, 37 days after slides were inserted. 650 ×

FIG. 9. Compact circular colony of spherical cells, probably Azotobacter. From vetch; slide in soil for 128 days. 1200 ×

FIG. 10. Colony of large, uniformly stained, vibrio-shaped cells. From vetch; slide in soil for 53 days. 1200 ×

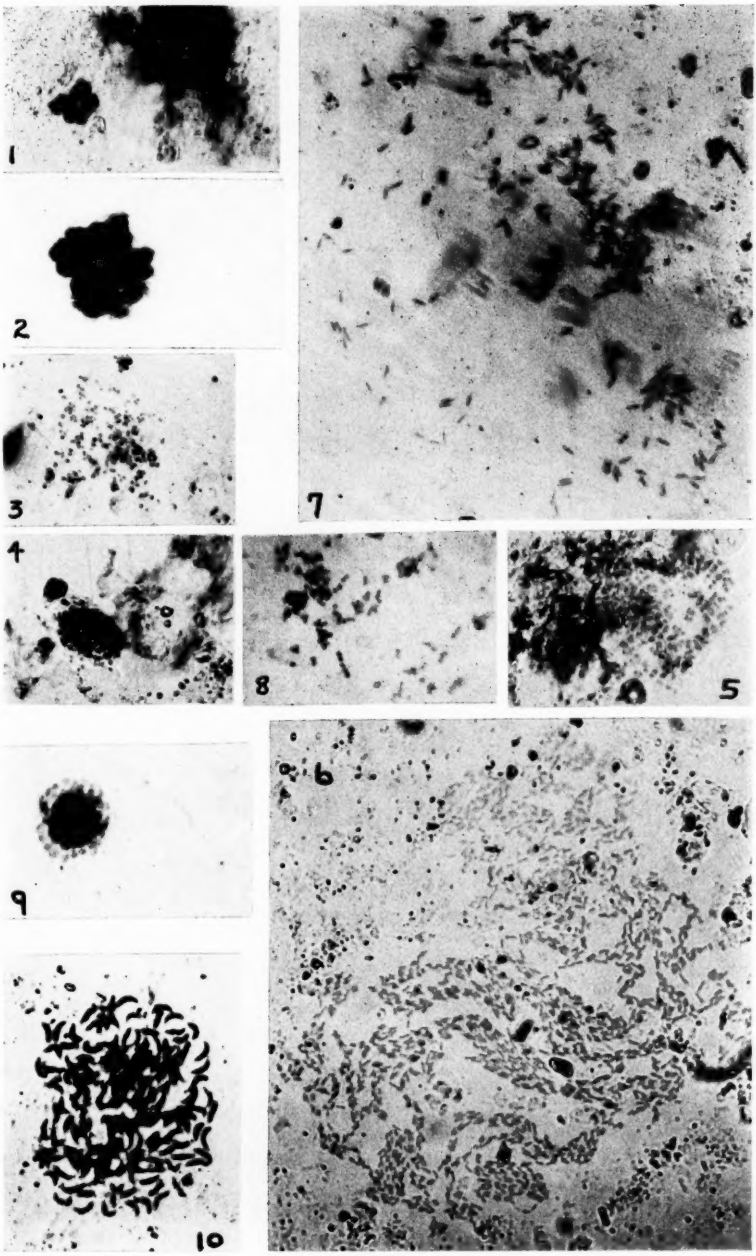
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PLATE 2

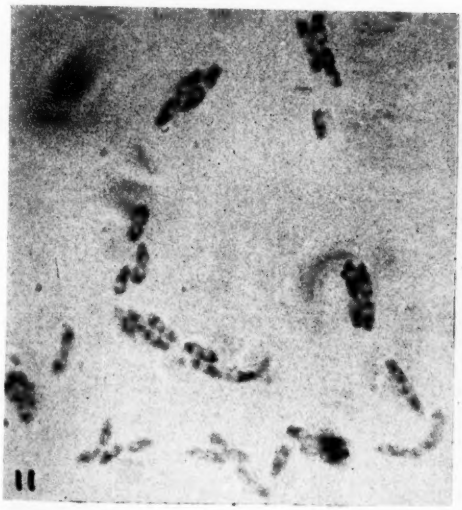
FIG. 11. Loose colony of large spore-forming rods. From rape; slide in soil for 37 days. 1200 ×

FIG. 12. Long slender spindle-shaped cells with central portion stained more deeply than the ends. Located about organic material and spreading over several microscope fields. Resembles Cytophaga. From maize; slide in soil for 40 days. 1200 ×

FIG. 13. Very long, lightly stained, tubular cells, each with a deeply stained rod-shaped body near one end (Bacteria?). From vetch; slide in soil for 53 days. 1200 ×

FIG. 14. Fragmented filaments of an actinomycete, showing conidia in short chains. Located near a bit of decomposing organic matter and spreading over several microscope fields. From rape; slide in soil for 37 days. 650 ×

FIG. 15. Fragmented actinomycete conidia. Deeply stained conidia and shadowy filaments of finer, lightly stained cells. Near decomposing organic matter. From rape; slide in soil for 37 days. 650 ×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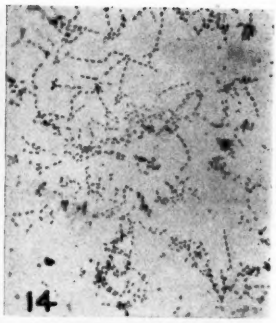
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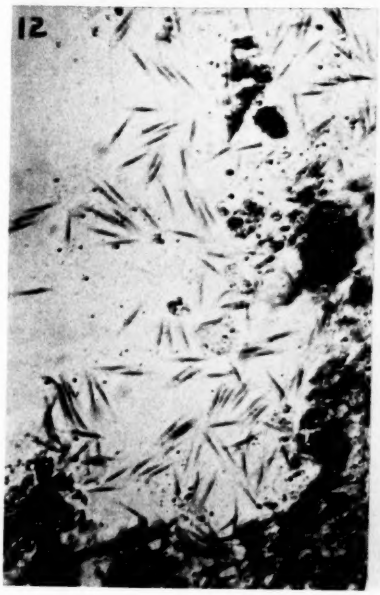
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PLATE 3

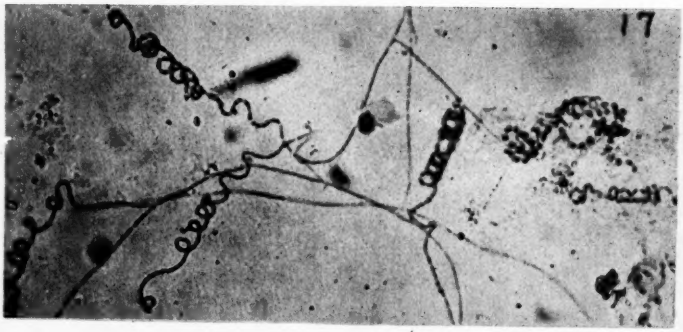
FIG. 16. Fragmented actinomycete filaments showing deeply stained conidia and shadowy outlines of finer, very lightly stained filaments. Portion of a very large group of cells spreading over many microscope fields in vicinity of decomposing organic matter. The conidia appear to be retained in a lightly stained sheath. From vetch; slide in soil for 53 days. 1200 ×

FIG. 17. Intact colony of actinomycetes showing branched filaments bearing open, spring like coils of conidia. The coil in the upper right hand section is composed of larger, more nearly spherical conidia than the others. From vetch; slide in soil for 53 days. 1200 ×

FIG. 18. Similar to figure 17. The loose coil near the top is composed of conidia which are nearly spherical and separated from one another. From vetch; slide in soil for 53 days. 1200 ×

FIG. 19. Chains of cells, believed to be actinomycete conidia, held together by thin strands. From maize; slide in soil for 40 days. 1200 ×

FIG. 20. Deeply stained germinated cells, believed to be actinomycete conidia, bearing fine branching filaments. From vetch; slide in soil for 53 days. 1200 ×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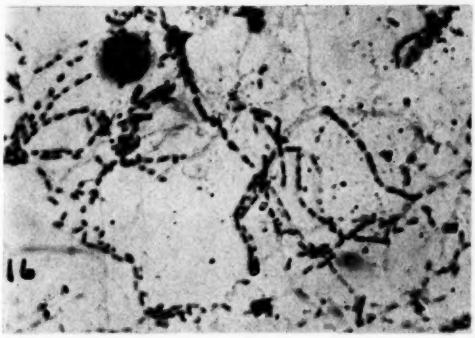
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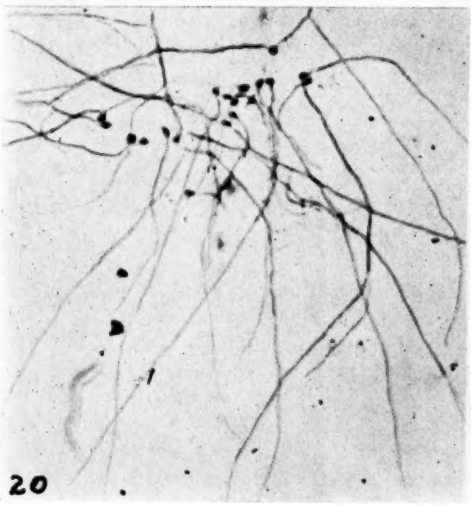
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PLATE 4

FIG. 21. Branched actinomycete filaments bearing open coils, some showing fragmentation into conidia. Similar to figures 17 and 18. From vetch; slide in soil for 53 days. 1200 ×

FIG. 22. Fungus spores, probably Fusarium. From fallow soil, 37 days after slide was inserted. 650 ×

FIG. 23. Cup-shaped brown fungus spores showing numerous striations. Shaped much like the cap to an acorn. From rape; slide in soil for 126 days. 1200 ×

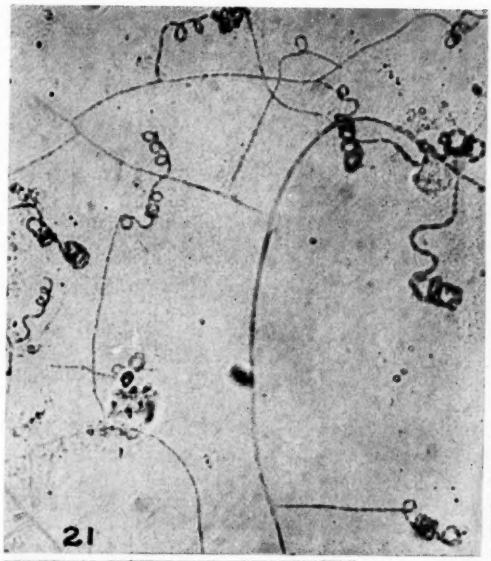
FIG. 24. Star-shaped cells with deeply staining edge and central body (fungus spores?). From vetch; slide in soil for 128 days. 1200 ×

FIG. 25. Sporangium of a Penicillium showing whorl of sterigmata. From vetch; slide in soil for 71 days. 1200 ×

FIG. 26. Sporangium of an unidentified fungus. Several of these arose from the same mycelium nearby. From vetch; slide in soil for 37 days. 1200 ×

FIG. 27. Pear-shaped spores born on short slender hyphae. The fungus may be related to Sporotrichum. Many such sporangia arose from the same mycelium. From rape; slide in soil for 37 days. 650 ×

FIG. 28. Large rectangular, vacuolated cells probably either Oidium or Monilia. In lower center is a large, brown, nearly spherical fungus spore. From rape; slide in soil for 37 days. 650 ×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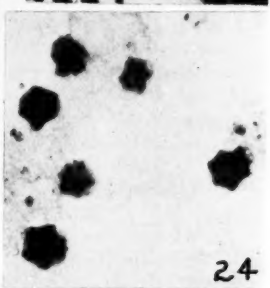
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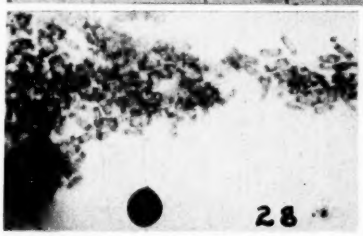
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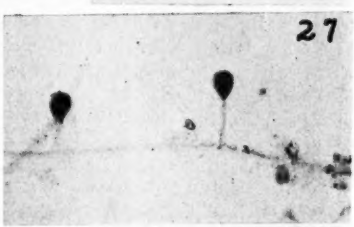
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PLATE 5

FIG. 29. Large fungus spores with five deeply staining bodies. Probably related to Helminthosporium. From rape; slide in soil for 37 days. 650 ×

FIG. 30. Dark brown, egg-shaped fungus spore. From vetch; slide in soil for 37 days. 615 ×

FIG. 31. Diatom skeleton, probably *Hantzschia amphioxys*, with pitted upper border. From maize; slide in soil for 40 days. 1200 ×

FIG. 32. Elongated diatom with deeply stained protoplasmic residue near one end. Borders were pitted and numerous striations were apparent under the microscope. From vetch; slide in soil for 128 days. 1200 ×

FIG. 33. Small, thin diatom skeleton with constricted middle region. Striations near ends run diagonally toward the center from each side. From maize; slide in soil for 40 days. 1200 ×

FIG. 34. Diatom with wide pitted borders. Stained deeply over a large portion of the cell. From vetch; slide in soil for 128 days. 1200 ×

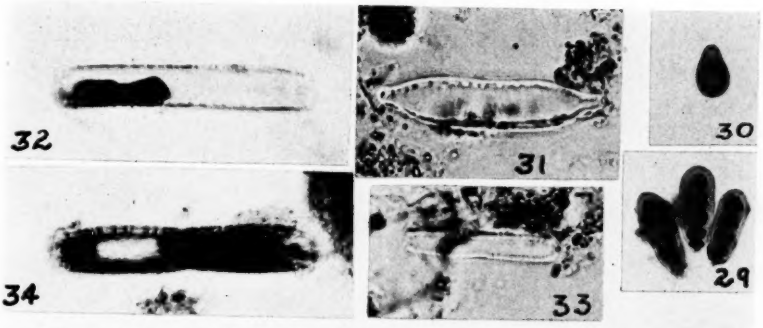
FIG. 35. Diatom showing ridged borders near both ends. Partly stained. From maize; slide in soil for 40 days. 1200 ×

FIG. 36. Chain of large spherical cells with deeply staining particles close to the cell walls (blue-green alga?). From vetch; slide in soil for 128 days. 1200 ×

FIG. 37. Insect parts; thin striated scales and spiny setae. From rape; slide in soil for 37 days. 650 ×

FIG. 38. Small animal being attacked by a fungus bearing single spherical spores on short hyphae; probably one of the Dematiaceae. The fungus filaments spread for a long distance from this location. From fallow soil, 37 days after inserting slide. 300 ×

FIG. 39. Localization of large numbers of coccoid bacteria about a small root which is undergoing decomposition. From vetch; slide in soil for 71 days. 1200 ×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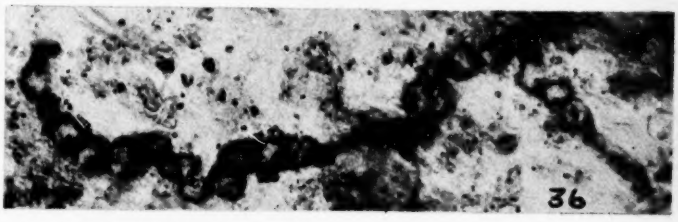
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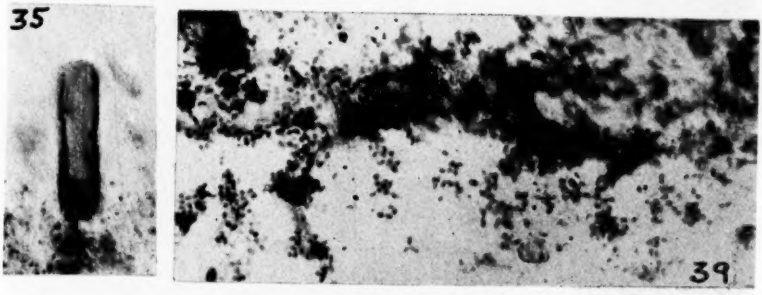
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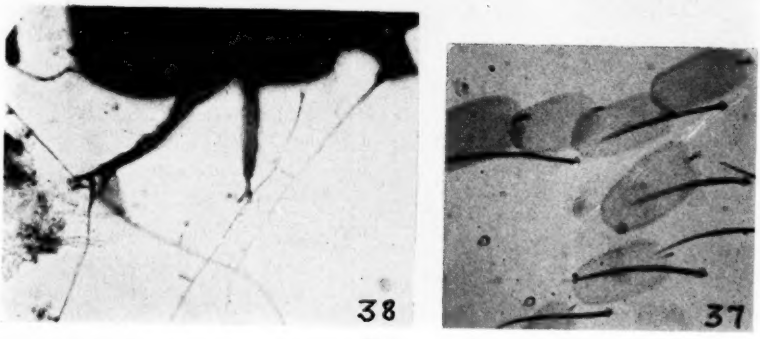
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PLATE 6

FIG. 40. Short rod-shaped bacteria developing about and upon a piece of root material. From vetch; slide in soil for 71 days. 1200 ×

FIG. 41. Edge of a large dense colony of bacilli growing upon organic material. A branched fungus filament showing septation is also apparent. From vetch; slide in soil for 53 days. 1200 ×

FIG. 42. Scattered, small, coccoid, bacterial cells developing along a fungus filament. From vetch; slide in soil for 71 days. 1200 ×

FIG. 43. Aggregates of tiny, lightly-stained, coccoid, bacterial cells developing about fungus mycelium. From rape; slide in soil for 37 days. 1200 ×

FIG. 44. Large dense colonies of tiny coccoid bacterial cells growing in contact with fungus mycelium. The individual bacterial cells are scarcely visible in these large colonies. From vetch; slide in soil for 37 days. 1200 ×

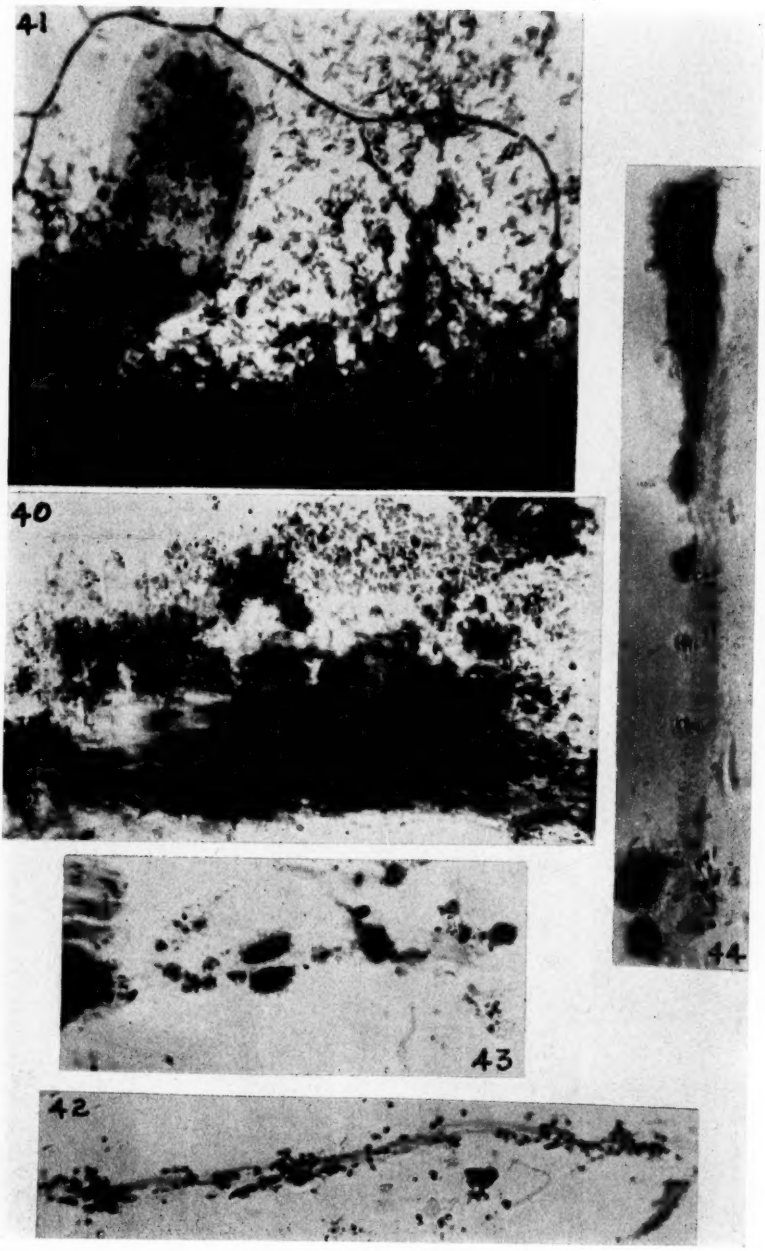
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PLATE 7

FIG. 45. Small colonies of coccoid bacterial cells growing along fungus mycelium. From vetch; slide in soil for 53 days. 1200 ×

FIG. 46. Considerable numbers of small, rod-shaped bacteria developing in contact with lightly stained fungus filaments. From vetch; slide in soil for 71 days. 1200 ×

FIG. 47. Root hairs with chains of very small, rod-shaped bacteria (upper center) and larger rods in contact with a root hair (bottom) and scattered at various other locations. From maize; slide in soil for 40 days. 1200 ×

FIG. 48. Ribbon-like root hairs invaded by numerous chains of tiny coccoid cells, probably bacteria. Large, branched, septate, fungus mycelium also developing about the root hairs. From vetch; slide in soil for 53 days. 1200 ×

FIG. 49. Terminal portion of a root hair showing numerous short rod-shaped bacteria in the form of a mantle. From mangel beet; slide in soil for 50 days. 1200×

FIG. 50. Short chains of actinomycete conidia in contact with an almost colorless root hair. A few lightly stained fine filaments can also be seen. From rape; slide in soil for 126 days. 1200 ×

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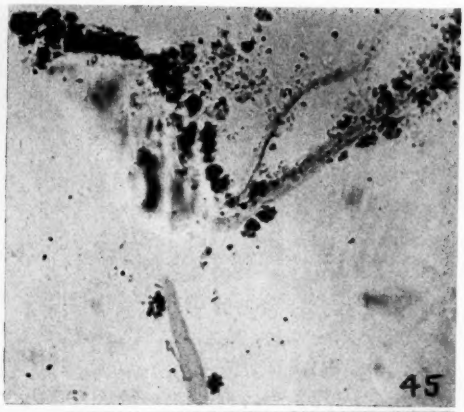
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PLATE 8

FIG. 51. Dense masses of tiny coccoid bacterial cells growing about a fungus filament. From barley; slide in soil for 53 days. 1200 ×

FIG. 52. Chains of small coccoid bacteria upon and within root hairs. From vetch; slide in soil for 71 days. 1200 ×

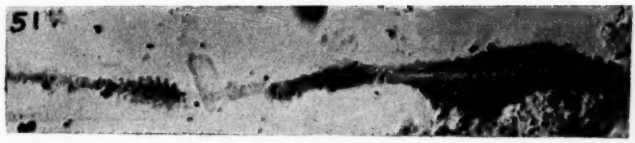
FIG. 53. Chains of small coccoid bacterial cells developing about root hairs. From rape; slide in soil for 126 days. 1200 ×

FIG. 54. A group of large ribbonlike root hairs supporting considerable numbers of chains of tiny coccoid bacterial cells which radiate from the root surfaces. From vetch; slide in soil for 53 days. 1200 ×

FIG. 55. Root hairs radiating from a large rootlet, with large numbers of rod-shaped bacteria developing about the root hairs and about the larger root. From mangel beet; slide in soil for 29 days. 650 ×

FIG. 56. Root formation similar to that in figure 55. Colonies of small rod-shaped bacteria as well as scattered cells about the root hairs. From mangel beet; slide in soil for 29 days. 650 ×

FIG. 57. Actinomycete filaments radiating from rootlet (right). The branched filaments bear typical, compact, coiled, sporulating bodies. From tomato; slide in soil for 23 days. 650 ×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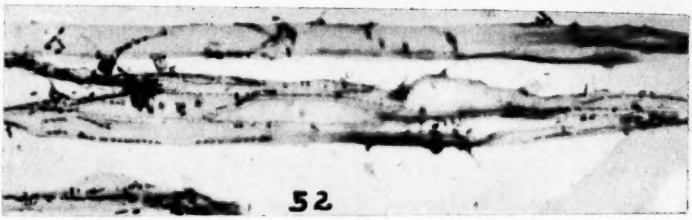
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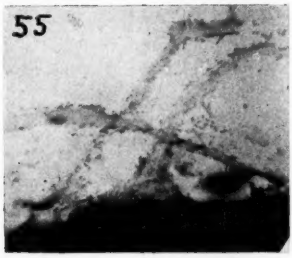
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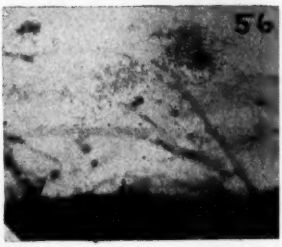
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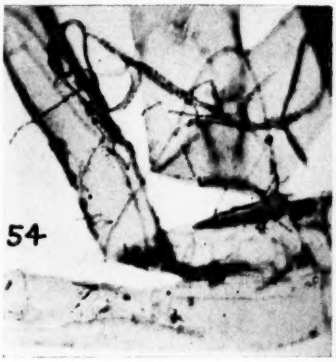
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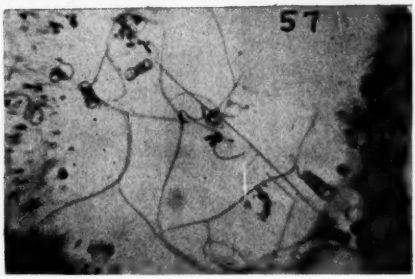
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PLATE 8

FIG. 51. Dense masses of tiny coccoid bacterial cells growing about a fungus filament. From barley; slide in soil for 53 days. 1200 ×

FIG. 52. Chains of small coccoid bacteria upon and within root hairs. From vetch; slide in soil for 71 days. 1200 ×

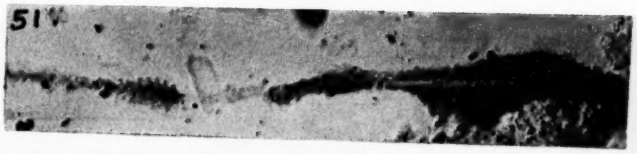
FIG. 53. Chains of small coccoid bacterial cells developing about root hairs. From rape; slide in soil for 126 days. 1200 ×

FIG. 54. A group of large ribbonlike root hairs supporting considerable numbers of chains of tiny coccoid bacterial cells which radiate from the root surfaces. From vetch; slide in soil for 53 days. 1200 ×

FIG. 55. Root hairs radiating from a large rootlet, with large numbers of rod-shaped bacteria developing about the root hairs and about the larger root. From mangel beet; slide in soil for 29 days. 650 ×

FIG. 56. Root formation similar to that in figure 55. Colonies of small rod-shaped bacteria as well as scattered cells about the root hairs. From mangel beet; slide in soil for 29 days. 650 ×

FIG. 57. Actinomycete filaments radiating from rootlet (right). The branched filaments bear typical, compact, coiled, sporulating bodies. From tomato; slide in soil for 23 days. 650 ×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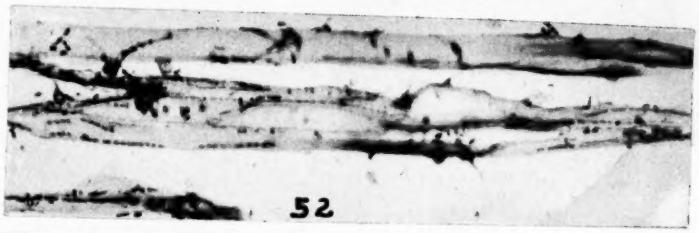
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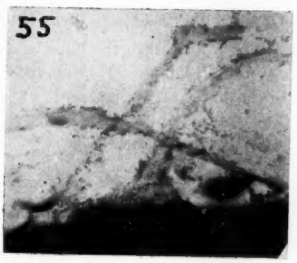
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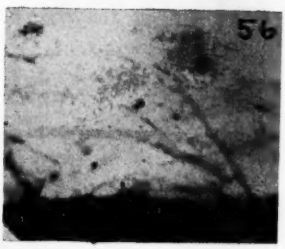
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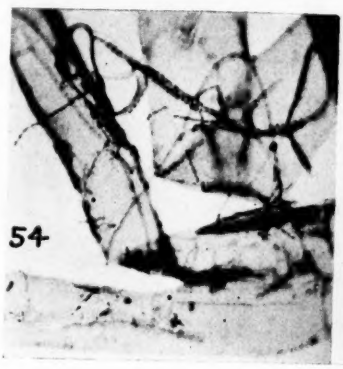
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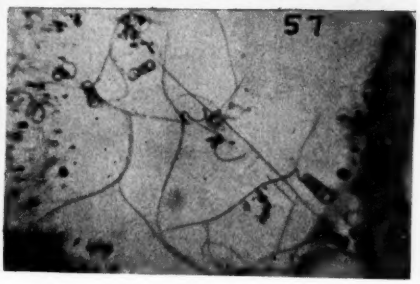
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PLATE 9

FIG. 58. Root hair with considerable numbers of deeply stained rod-shaped bacteria in colonies and in short chains along the surface. Small spherical colonies of bacteria also a short distance from the root. From maize; slide in soil for 40 days. 1200 ×

FIG. 59. Colonies of rod-shaped bacteria about a clear zone from which a rootlet may have been detached during preparation of the slide. From mangel beet; slide in soil for 29 days. 650 ×

FIG. 60. Root hair with scattered tiny coccoid cells (upper left, on and about the root hair) and radiating, branched, actinomycete filaments. From maize; slide in soil for 40 days. 1200 ×

FIG. 61. Root hairs undergoing extensive attack by bacteria and actinomycetes. Fine branched filaments and larger, more deeply staining rods. Root hairs almost entirely decomposed. From vetch; slide in soil for 71 days. 1200 ×

FIG. 62. Small portion of a large rootlet, which has been almost completely decomposed, with profuse development of chains of small coccoid cells, probably bacteria. From vetch; slide in soil for 53 days. 1200 ×

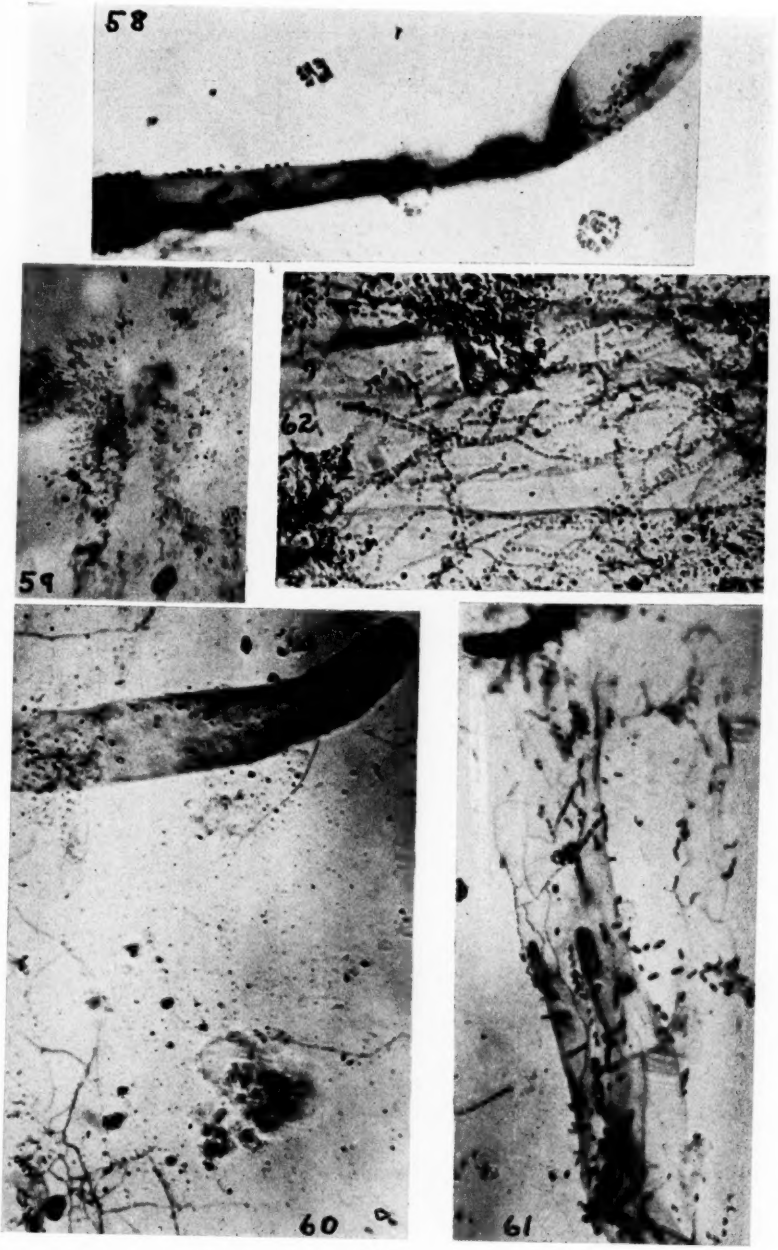
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PLATE 10

FIG. 63. Root hairs almost entirely decomposed and various scattered microbial cells, including rod-shaped bacteria, actinomycete conidia, and fungus spores (left center). From vetch; slide in soil for 71 days. 1200 ×

FIG. 64. Advanced stage of decomposition of root hairs showing abundance of rod-shaped bacteria, actinomycete filaments, short pieces of fungus mycelium, and a few fungus spores. From vetch; slide in soil for 71 days. 1200 ×

FIG. 65. Portion of a large root with a dense colony of small coccoid bacterial cells upon it and another loose aggregate a short distance away. From rape; slide in soil for 126 days. 1200 ×

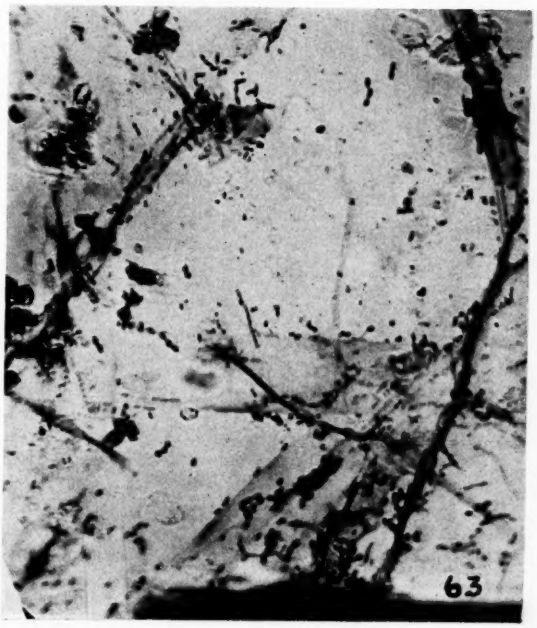
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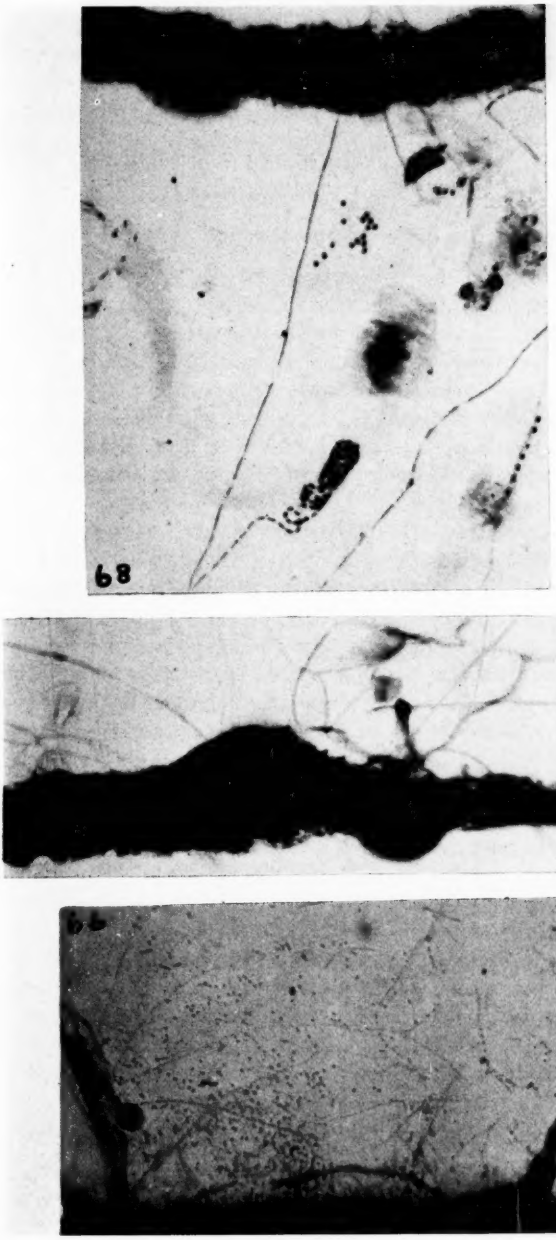
PLATE 11

FIG. 66. Scattered bacterial cells and actinomycete filaments about a large root. The large projection at the lower right is the head part of a nematode which was upon the root surface. From vetch; slide in soil for 53 days. 1200 ×

FIG. 67. A rootlet covered with great numbers of rod-shaped bacteria (or actinomycete conidia?); swellings are formed by the large accumulations of cells. Shadowy outlines of actinomycete filaments can be seen about the rootlet. From vetch; slide in soil for 37 days. 1200 ×

FIG. 68. Rootlet completely covered with rod-shaped bacterial cells (actinomycete conidia?). Filaments of an actinomycete extend from the rootlet, one of which bears a spiral coil completely fragmented into conidia. From vetch; slide in soil for 37 days. 1200 ×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SOME THERMAL PHENOMENA IN A SELECTED HAWAIIAN SOIL[1]

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Hawaii Agricultural Experiment Station

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Recent attempts to use freezing processes for the determination of soil moisture constants as suggested by Schofield (10), by Schofield and Da Costa (11), and by Bouyoucos (3) have focused attention upon certain thermal effects which closely circumscribe the use of freezing procedures with local soils. The history of the sample of a selected local soil prior to the determination of the freezing point depression has been primarily responsible for the value obtained; without most careful control of the factors involved in moisture movement in the prepared sample, concordant results have been unobtainable.

Since freezing is a drying process and is useful only in locating points on the drying arm of the so-called "hysteresis loop," samples for freezing were prepared by drying samples which had been wetted to about the moisture equivalent by Bouyoucos' (2) method until moisture contents suitable for freezing point determinations had been reached. Usually this was done by dividing a 75-gm. lot of soil, wetted to the moisture equivalent, among five large watch glasses and exposing them for varying periods on the laboratory table. All samples were frequently stirred during the process of drying.

Difficulties were soon encountered. When the freezing point depressions obtained from any one series were expressed as corresponding pF values, according to the Schofield convention, and plotted against soil moisture contents, a smooth curve resulted in about the position expected from determinations of moisture equivalent and permanent wilting percentage. Upon repetition a second smooth curve might be obtained, but ordinarily the second curve was not coincident with the first. It soon became apparent that the air conditions in the laboratory were at least partially responsible for this unexpected result. Samples brought down to suitable moisture contents on a hot, dry day exhibited a much lower pF, at a given moisture content, than would be found if the samples were prepared during a colder day with relatively high humidity. This effect was much exaggerated when samples were prepared by short-time drying in a low temperature oven.

It is the purpose of this note to call attention to this unexpected result

[1] Published with the consent of the director of the Hawaii Agricultural Experiment Station.

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and to point out other evidences of internal energy relationships in incomplete and superficially dried soils.

THE SOIL USED

Studies on the thermal relations mentioned have been restricted to a single soil representative of sugar cane and pineapple areas on Oahu. This soil is the result of the residual chemical weathering of Hawaiian basic lava under conditions of high temperature and moderate rainfall. Although granular when air dry, the soil can be dispersed to such an extent that virtually all of it stays in suspension in water for long periods. Recent mechanical analyses by the Bureau of Chemistry and Soils, U. S. Department of Agriculture, have indicated that the soil should be classified as clay although it feels and acts like a silt loam soil in the field.

The soil is relatively rich in iron and aluminum oxides and poor in silica. Fusion analysis of the colloidal separate, obtained by conventional means, gave the following results:

	Per cent
Ignition loss	17.3
SiO_2	29.8
Al_2O_3	32.1
Fe_2O_3	19.3

The moisture equivalent as determined by the centrifuge is 30.5 per cent, and the permanent wilting percentage, determined by the growth of plants, is 23.5 per cent. With frequent irrigations and adequate mineral fertilization the soil is highly productive.

Heat of wetting determinations upon the freshly oven-dried soil give a value of 3.41 calories per gram. Gile (7) gives a heat of wetting of about 4 calories per gram for soil colloid with the silica-sesquioxide ratio indicated above. The highly colloidal nature of the soil used is evident.

HEAT EVOLUTION IN SUPERFICIALLY DRIED SOIL

In order that conditions might parallel those existing in the usual freezing-point depression measurements as closely as possible, 15-gm. lots of soil with different drying histories were supercooled to $-1.8°C$. In order to accomplish this the loaded tube, carrying a Heidenhain thermometer, was clamped into an ice-salt bath and allowed to remain without jarring until the temperature had fallen to the desired point. It was then quickly moved to a large Dewar flask containing shaved ice and distilled water. In most cases, and with the exceptions noted, the temperature immediately rose to a temperature significantly above that of the ice water bath and then fell slowly to it. The magnitude of this heating is shown in table 1.

It would seem logical to infer that heat was being generated within the soil, this effect probably being due to the internal readjustment of moisture. If

this be true the temperature rise in the soil mass finds logical explanation in the assumption that such evolution is at a rate faster than that by which heat can be carried away through the soil and through the glass in view of the small thermal gradient.

Another example of the significance and duration of this internal source of heat is to be noted in another sample of stock soil heated to 110°C. for 10 minutes and supercooled to −1.8°C. as above. In this case the tube was thrust into an ice water bath at 0°C. Frequent temperature readings were taken in the soil and in the external bath. After 9 minutes the soil temperature exceeded that of the bath by 0.15°C. Although this difference became less with time, a significant difference was noted after 85 minutes. At this point the soil was removed, and the temperatures inside the tube and in the bath became equal after 5 minutes.

TABLE 1

Temperatures attained by soil masses with different drying histories when supercooled to −1.8°C. and then put into bath at 0°C.

TEST NUMBER	DRYING HISTORY	MAXIMUM TEMPERATURE
		°C.
1	Stock soil at 26.5 per cent moisture, dried all night in oven at 35°C. with fan running	+0.005*
2	Stock soil dried in sun for 10 minutes with frequent stirring	+0.30
3	Soil from test 1 dried in sun as in test 2	+0.14
4	Stock soil in oven at 110°C. for 10 minutes	+0.30

* The departure of this value from zero is of doubtful significance.

Ordinarily the freezing point of water in a soil lies below 0°C. because of its content of salts and the forces by which the water is bound to the colloid.

It is evident not only that significant amounts of heat were evolved in the soil but that the rate of heat evolution for more than an hour was essentially equal to the rate at which heat was lost through the walls of the container under the influence of a thermal difference of about 0.10°C.

It will be recalled that this procedure varies from the usual freezing-point depression measurement only in the fact that in the latter case freezing is begun when desired with a quick twist of the thermometer. With most soils, particularly soils in which perfect moisture equilibrium has been effected, it it probably true that the characteristic sudden rise in temperature is due to the formation of ice. With soils lacking uniformity of moisture distribution it would appear that the rapid increase in temperature might be modified by the phenomenon which has been noted. If this be true it is probable that the temperature reported as the freezing point in a soil lacking uniformity of moisture distribution would be greater than it should be and the freezing point depression less than it should be for the average moisture content of the

sample. It will be noted that this explanation tends to correct the discrepancies in freezing-point depression measurements which have been reported.

Further evidence that the heat source already noted is correlated with the heterogeneous moisture distribution within the sample and not with the moisture content itself is provided in table 2. Samples of stock soil were partially dried in an oven held successively at 40, 50, 60, 80, and 100°C. Individual samples were withdrawn from each series after the time intervals noted in table 2. Samples were at once placed in standard freezing tubes, supercooled to −1.8°C., and instantly put into a Dewar flask containing shaved ice and water.

Temperature within the soil mass was observed at half-minute intervals. The maximum obtained in each case is reported in table 2.

TABLE 2

Increases of temperature in soil masses brought to varying moisture contents by different drying procedures

TIME IN OVEN	40° SERIES		50° SERIES		60° SERIES		80° SERIES		100° SERIES	
	Moisture	Δt	Moisture	Δt	Moisture	Δt	Moisture	Δt	Moisture	Δt
minutes	*per cent*	°C.	*per cent*	°C.	*per cent*	°C.	*per cent*	°C.	*per cent*	°C.
3	....	....	....	....	....	....	....	....	20.4	0.09
5	25.5	0.04	24.8	0.02	22.5	0.07	19.3	0.10	....	....
6	....	....	....	....	....	....	....	....	13.8	0.04
10	....	....	....	....	18.5	0.11	12.0	0.03	....	....
12	22.7	0.04	22.1	0.09	....	....	....	....	....	....
15	....	....	....	....	15.1	0.10	8.9	0.05	....	....
20	20.9	0.04	17.7	0.10	13.4	0.11	....	....	....	....
30	19.5	0.07	16.2	0.06	7.0	0.09	1.7	0.02	....	....
40	12.6	0.03	9.7	0.09	....	....	....	....	....	....
60	....	....	....	....	4.9	0.03	....	....	....	....

The temperature increases noted in table 2 provide additional evidence that moisture readjustment within the particular soil studied provides a source of heat of surprising magnitude. Although the information furnished does not lend itself readily to quantitative interpretation, it is to be noted that some rough sequence is evident. The maximum temperature difference of +0.11°C. is to be noted in the 60° series with consistent decreases in this value with hotter and colder drying. Moreover, as might be expected, the maximum temperature increase is reached after progressively shorter times as the drying temperature is increased.

Definite lack of correlation between gross moisture content as determined by conventional methods and the increase of temperature over the surroundings draw attention once more to the fact that lack of uniformity of moisture within the sample and not gross moisture content must be responsible for the observed energy change. It is interesting to note that such temperature

increases are not observed when oven-dried soils are used as well as moist soils which have had ample time to come to moisture equilibria.

HEAT EVOLUTION ON MIXING SOILS OF DIFFERENT MOISTURE CONTENTS

The argument that heat is evolved when soil particles of different moisture contents are brought into close contact suggests that the phenomenon should be observed after a simple mixing of such soils. A large sample of the standard soil was oven dried and brought to a specified temperature, departing but slightly from that of the room, in a tightly stoppered bottle suspended in a water bath. The same bath carried a bottle of moist stock soil which had been carefully mixed 3 months prior to the beginning of the present tests. It is probable that uniform moisture distribution had been attained in the moist lot. In the tests summarized in table 3, samples of each of the two lots were

TABLE 3

Heat evolution on mixing varying quantities of moist and of oven-dry soil

TEST NUMBER	SOIL USED		Δt	CALORIES*	MOISTURE	WATER MOVED†
	Wet	Dry				
	gm.	*gm.*	°C.		*per cent*	*gm.*
1	35	5	0.65	10.9	20.1	1.01
2	30	10	1.75	28.0	16.6	1.70
3	25	15	2.24	34.2	13.6	2.02
4	15	25	2.55	35.7	7.8	1.91
5	10	30	4.05	47.0	5.0	1.51
6	5	35	4.20	52.9	2.3	0.85

* Based on specific heat of 0.3 for oven-dry soil involved.

† Based on assumption that decrease in moisture content of wet component measures total amount of water moved.

quickly weighed, mixed as quickly as possible on a hard filter paper, and thrown into a small Dewar flask provided with a rubber stopper carrying a certified thermometer graduated to 0.1°C. Temperature readings were begun immediately, toward the end of obtaining the maximum. The thermal insulation was so complete that the fall of temperature after the maximum had been reached was extremely slow. It is probable that losses of heat by radiation, during the short period of the rise of temperature, were negligible.

After the maximum temperature was noted in each mixture, the moisture content of the mixed soil was determined by oven drying. The actual moisture contents of all mixtures were close to those computed from the known moisture contents of component parts of the mixtures.

For convenience, 40 gm. of mixed soil was used in each test. With the flask used, this provided complete immersion of the thermometer bulb.

Details of the observations are shown in table 3.

SOIL TEMPERATURES AS INFLUENCED BY WETTING AND DRYING UNDER THE INFLUENCE OF AN AIR STREAM

It has been suggested that the source of the heat noted was the equalization of moisture in a heterogeneous system of different moisture contents. Another aspect of the same conception is the familiar evidence of heat evolution when dry soil is placed in water. In the conventional procedure heat is evolved; under other conditions a cooling effect should be noted.

In order that this possibility might be tested 100 gm. of freshly dried stock soil was placed in a Dewar flask with a 3-hole stopper. In two of these holes were inserted glass tubes, one extending below the soil surface and the other ending just below the stopper. A thermometer, the bulb of which was immersed in the soil mass, extended through the third hole.

A conventional assembly of saturating tubes and dehydrating agents permitted an immediate change of humidity of the air stream passing through the soil under the influence of an air pump. The air stream in one path was saturated when it reached the soil; that in another path was practically dry. Traps in each line permitted observations of temperatures immediately prior to introduction of the air to the soil. A tube connected to the outlet port on the soil flask discharged under water, giving evidence of the fact that the air stream was moving, as well as providing means for judging the volume of air passing per unit of time.

When dry soil was used, a static run of 25 minutes showed no change of soil temperature. When dry air was run through the system for 35 minutes no change of temperature was apparent. When saturated air was substituted for dry air the soil temperature rose from 26.95 to 30.95°C., an increase of 4°C., in 30 minutes. Because of outside conditions the temperature of the air stream actually fell during this period. When dry air was introduced the temperature fell to 29.10°C. within 30 minutes. Another change to saturated air drove the soil temperature up to 31.75°C. within 15 minutes.

A dry soil sample which had been brought to a moisture content of 11.9 per cent by use of an atomizer showed entirely different responses to variations in air-stream humidity. In this soil, when dry air was used, the fall of temperature was extremely rapid, a 3.4°C. depression being noted in 20 minutes. Temperature increases under the influence of the wet air stream were much slower than the decreases under the influence of the dry air.

CONVENTIONAL HEATS OF WETTING WITH SOILS AT VARYING MOISTURE CONTENTS

Except for the recent work of Hoseh (8), who used soil dried to equilibria at varying temperatures, conventional heat of wetting determinations are ordinarily made on oven-dried samples. It would appear that the same phenomenon should be apparent with soils at other moisture contents, although in smaller degree.

Small lots of oven-dried soil were brought to predetermined moisture con-

tents by spraying water from an atomizer over a constantly changing soil surface. When the required amount of water had been applied, the samples were placed in large tubes and turned in an end-over-end shaker for 30 minutes. The samples were then divided into two parts, one of which was used for the determination of moisture content and the other for subsequent heat of wetting measurements. Materials for subsequent use were carefully weighed into small thin-walled tubes and stored in the dark, with occasional shaking for 48 hours.

Heat of wetting determinations were made in a vacuum flask in place of a calorimeter. Repeated observations indicated that radiation losses under the temperature differences involved were negligible. In all tests, 100 gm. of distilled water was used. Results are shown in table 4.

TABLE 4

Heats of wetting with stock soil at varying moisture contents

TEST NUMBER	SOIL, OVEN-DRY BASIS	CALORIES EVOLVED	CALORIES PER GRAM	MOISTURE
	gm.			*per cent*
1	12.75	43.47	3.41	0
2	14.30	41.76	2.92	0.89
3	15.69	34.36	2.21	1.47
4	11.15	6.25	0.56	6.82
5	15.62	11.62	0.74	6.44
6	14.54	6.35	0.44	10.55
7	16.08	2.14	0.13	14.66

DISCUSSION

Although the results reported in this paper were obtained from a series of short tests of inadequate scope, they do suggest another approach to the study of internal energy relationships of soils. Unfortunately, many of the procedures used to indicate the evolution of heat when dry soils are wetted, either through contact with wetter soil or by a stream of saturated air, are of limited quantitative use. For example, a temperature increase in a dry soil mass after the passage of saturated air can be convincingly reported only in terms of calories per gram of soil when one is assured of uniform moisture content in the soil mass or can demonstrate the gradient by which moisture content falls from the bottom of the air inlet tube to the drier soil removed from it. When a moist soil is mixed with a dry one, significant but indeterminate losses of the heat generated by the mixing should be expected during the process of mixing. Moreover, the temperature attained by the thermometer when the mixture is finally admitted to the Dewar flask is the temperature in the immediate vicinity of the bulb. Because of lack of uniformity in the quickly mixed sample, the observer cannot estimate how uniform this temperature may be.

Considerable qualitative significance may be attached to the observations, although the observations reported have only added visual demonstration to the energy relationships already suggested by Buckingham (4), by Gardner (6) and his co-workers, and more recently by Schofield (10).

Using the pF convention of Schofield for convenience of expression, it seems evident that any process, in the list of those reported, which decreases the pF of a soil liberates heat, whereas any process which increases the pF absorbs heat. A special case of this general principle has long been recognized in the heat of wetting. Here oven-dry soils are added to water. From definition, the pF of the soil falls from about 7.0 to 0. Only recently (8) has there been any effort to measure the heat of wetting with soil wetter than oven dry. It is evident, however, that in a definite mixture of damp soil and dry soil one fraction must be dried and the other wetted as moisture equilibrium is reached. It would follow that as equilibrium is reached one fraction tends to cool the mixture while the other adds heat. From the shape of the force-moisture content curve, however, regardless of the units in which it is presented, it is evident that adding unit mass of water to dry soil is more significant in net heat evolution than withdrawing unit mass of water from damp soil. It should also be noted that one component of this mixture is on the so-called "drying arm" (12) whereas the other is on the wetting arm of the force-moisture content curve. Although Edlefsen (5) has questioned the significance of this distinction, the slopes of the energy-moisture curves for many soils during wetting and drying regimes (10, 12) indicate that greater emphasis should be placed on the energy relations in the wetting sample than on those in the drying sample when unit mass of water is involved.

Table 3, giving heat evolved in calories from the mixing of varying quantities of oven-dry soil and soil at 23.6 per cent moisture, provides qualitative examples of the principle already suggested. In test 1, 5 gm. of oven-dry soil (at pF 7) was mixed with 35 gm. of soil at pF 4.2. There is evidently a potential in favor of movement into the dry soil, but here only a small amount of water need be moved to effect equilibrium, in view of the small quantity of dry soil involved. We consequently have a relatively small evolution of heat. When relatively large quantities of dry soil are used we have a quite different effect. Here again only a small amount of water need be moved to bring about equilibrium. The pF of the dry soil falls from 7.0 to about 6.0, but the quantity of dry soil is great. As has been indicated, attempts to force such results into quantitative aspects cannot be successful.

The same vague relationship is to be noted in results coming from soils wetted and dried under the influence of air streams of varying humidities. The temperature of the cooled oven-dry soil at about pF 7 showed no change when dry air was used but rose rapidly when saturated air was used. In view of the increased moisture content due to this treatment, subsequent treatment by dry air resulted in a fall of temperature. The relationship of these observations to the general principle which has been suggested is evident, but here quantitative interpretation is even more difficult.

The only possibility in quantitative study, with the present technic, lies in the interpretation of the heat of wetting results. Several series of observations aimed toward obtaining moisture equilibria over sulfuric acids of varying concentrations resulted in a pF-moisture content relationship as shown in curve A, figure 1. In the tests involved the standard soil was used, and the necessary conditions such as constant temperature and absolute darkness (9) were provided. It is evident that curve A in figure 1 represents the "wetting arm" of the curve.

In figure 2, heats of wetting in calories per gram of soil are plotted against percentages of moisture (Table 4).

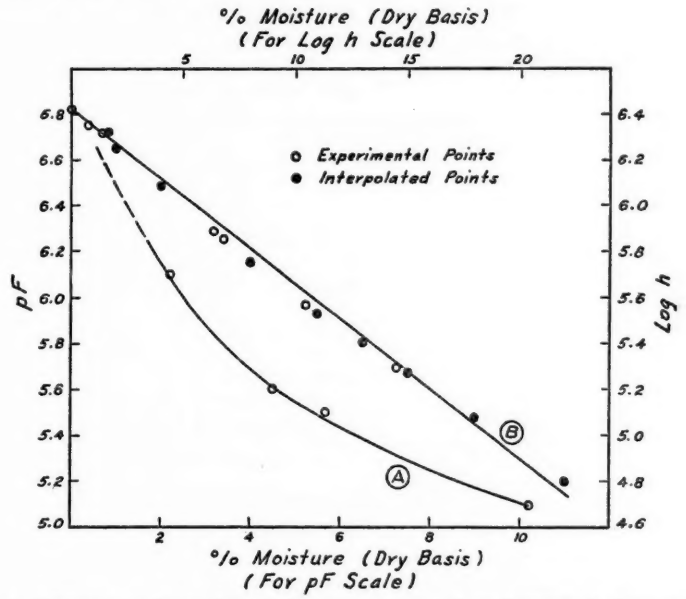

FIG. 1. MOISTURE OF STOCK SOIL AS RELATED TO THE pF BY SCHOFIELD'S CONVENTION (CURVE A) AND TO A FUNCTION OF THE HEAT OF WETTING (CURVE B)

Such curves are immediately suggestive of an exponential form. When the equation

$$H = 3.41\epsilon^{-0.237P}$$

where H = heat of wetting in calories per gram of soil and
P = per cent moisture (dry basis).
is used the computed values of H agree close with the observed values. Points to the right of 14.66 per cent are obtained by extrapolation.

It is evident that the area under the curve and to the right of any ordinate is proportional to the energy released in bringing soil, at the moisture content

represented by that ordinate, to the condition of saturation. Alexander and Haring (1) have used the same graphical device in studying the energy relations in soil colloids. The areas on an arbitrary scale, represented by the moisture contents used in table 4 as well as for interpolated points, are given in table 5.

Although the scale used in measuring areas reported in table 5 is arbitrary, the proportionality constant introduces no difficulty. Column 5 in table 5 gives the total energy in terms of gm.-cm., liberated as heat in each test. Since we assume that no heating is perceptible when the original moisture content exceeds 24 per cent, we have an expression for the mass of the active

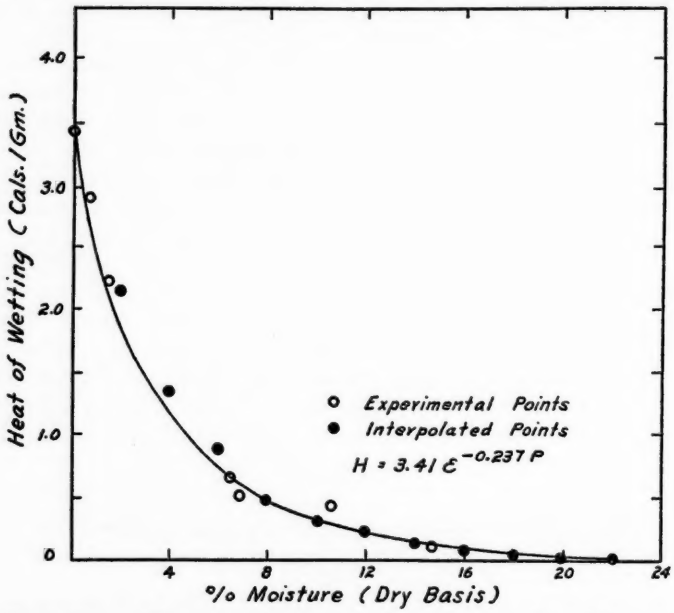

FIG. 2. HEAT OF WETTING, IN CALORIES PER GRAM, FOR STOCK SOIL AT VARYING MOISTURE CONTENTS

water per gram of soil as given in column 3. Dividing the total energy expressed in gm.-cm. by this weight gives an "equivalent height" in centimeters called h in column 6. The analogy between log h and the conventional pF is evident. Log h is plotted against percentages of moisture in curve B, figure 1.

The straight line formed by these points indicates another exponential form. For the particular soil used

$$\log h = 6.42 - 0.078\, p$$

where h = height of water column as determined in table 5 and p is the percentage of moisture. It is probable that the coefficient for p varies with the

character of the soil used, but with the limited data at hand this possibility cannot be explored.

Another interesting comparison focuses attention upon the evident correlation between the conventional pF expression and the energy of wetting.

TABLE 5

Energy released in saturating stock soil at varying initial moisture contents

MOISTURE	MOISTURE INCREASE*		AREA UNDER CURVE, ARBITRARY UNITS	GM.-CM. $\times 10^4 \times C$†	EQUIVALENT HEIGHT $(h) \times 10^4 \times C$	LOG h + LOG C
		Active water per gm. soil				
per cent	*per cent*	*gm.*				
22	2	0.02	0.150	0.128	6.4	4.81
18	6	0.06	0.850	0.724	12.0	5.08
15	9	0.09	1.975	1.683	18.7	5.27
14.68‡	9.36	0.0936	2.150	1.832	19.5	5.29
13	11	0.11	3.281	2.794	25.3	5.40
11	13	0.13	5.231	4.392	33.8	5.53
10.55‡	13.45	0.1345	5.805	4.946	38.6	5.57
8	16	0.16	10.639	0.965	56.6	5.75
6.82‡	17.18	0.1718	14.201	12.098	70.4	5.85
6.44‡	17.56	0.1756	15.578	13.270	75.6	5.89
4	20	0.20	28.782	28.750	122.5	6.09
2	22	0.22	45.907	39.111	177.9	6.25
1.47‡	22.53	0.2253	54.597	46.779	207.1	6.32
0.89‡	23.11	0.2311	61.643	52.517	227.1	6.36
0‡	24	0.24	75.068	63.955	266.2	6.42

* It is here assumed that the evolution of heat at 24 per cent is negligible.

† C is proportionality constant; 1 cal. = 4.26×10^4 gm.-cm.

‡ Experimental points.

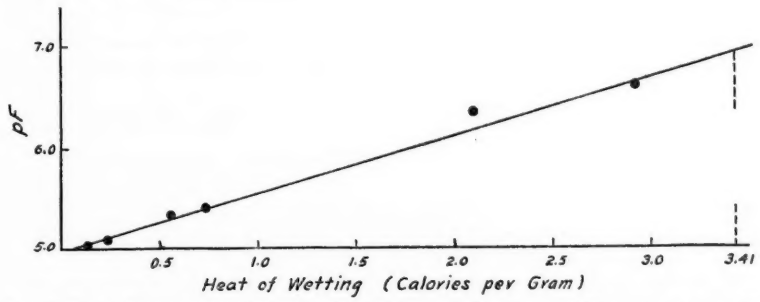

FIG. 3. HEAT EVOLUTION AT VARYING SOIL MOISTURE CONTENTS AS RELATED TO THE pF OF THE SOIL AT THE SAME MOISTURE CONTENTS

Here the pF values were obtained from desiccator studies which have been mentioned, and the corresponding energy relations were lifted from table 5. Corresponding data are plotted in figure 3. Again we note the typical exponential form. If work with one soil can be considered indicative, it is evident

that a correlation exists between the value of the pF as found by holding samples of oven-dry soil over sulfuric acid and the heat of wetting obtained by saturating samples of soil at that moisture content.

SUMMARY

Attempts to determine the permanent wilting point of local soils by freezing point depressions indicated that, with one local soil at least, the freezing point depression was significantly affected by the degree of uniformity of moisture distribution within the sample. Further study gave some evidence that a measurable evolution of heat occurs when relatively dry soils are brought into close proximity to wetter soils.

Although this principle has been demonstrated qualitatively in several ways, only one of them provides a quantitative approach with the present technic. There is some evidence that the conventional heat of wetting, with soils at initially varying moisture contents, is closely correlated with the moisture potential of the sample.

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THE NATURE OF POTASH FIXATION IN SOILS[1]

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Soil investigators agree that when soluble potash fertilizers are applied to soils the potassium may be retained in either or both of two forms; namely, the readily available form and the difficultly available form. That which enters the base exchange complex and remains exchangeable is essentially the readily available form, and that which is fixed so that it cannot be extracted with salt solutions or dilute acids is the difficultly available form. The nature of this latter type of fixation has been studied by a number of investigators, but their findings still leave much that is unexplained. It was the purpose of the present investigation to obtain additional information on this subject.

HISTORICAL

In 1934, Volk (2), reported on investigations pertaining to potassium fixation in soils in difficultly available form. For a review of the literature pertaining to this subject, the reader is referred to this report. Volk found that when a mixture of soil and water containing a soluble postassium salt was evaporated to dryness at room temperature, some of the potassium was fixed in difficultly available form. Alternate wetting and drying of this mixture at 70°C., caused a large portion of the potassium to be fixed in this form by certain soils. If the soils containing the soluble potassium salt were allowed to stand in a moist condition, fixation, at most, was very slow. The colloid fraction of the soil fixed much more potassium than did the other separates. His data indicate that the potassium is not just mechanically held, but enters into a chemical reaction with the colloid. Previous leaching of the soil with 1 *N* HCl reduced the fixation, and leaching with 1 *N* Na_2CO_3 increased the fixation. Synthetic mixtures consisting of alumina gel, silica gel, calcium hydroxide, and sand did not fix potassium. Chemical, mineralogical, and X-ray studies of Hagerstown silt loam from two plats of the Pennsylvania Station field, one not having received potash as fertilizer, and the other having received 3,158 pounds of

[1] Part of a thesis submitted to the faculty of the University of Wisconsin in partial fulfillment of the requirements for the degree of doctor of philosophy. This investigation was supported in part by a fellowship grant from the N. V. Potash Export My., Inc., New York, N. Y. Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

[2] The writer expresses his appreciation for the many helpful suggestions and criticisms tendered by E. Truog, under whose general direction this work was done.

potash, led to the concludion that muscovite had been formed in the latter, thus accounting for the fixation of the potash. A reaction between the colloidal silicates of the soil and the potassium added, forming muscovite as the end product, was believed to have taken place.

POTASH FIXATION BY SOIL MINERALS

In studying the factors responsible for potash fixation in soils in difficultly available form, the capacity of the more common soil minerals to fix potash was first investigated. Volk (2) tested a number of common soil minerals for their fixing capacities and found that none of those investigated would fix sufficient quantities to account for the amount fixed by soils.

Fixation by powdered minerals and natural rock products

Since Volk (2) had concluded that potash is fixed as muscovite, it was thought that some of the potash deficient muscovites might react with the added potassium. Several different samples of muscovite containing from 9.3 to 10.8 per cent potash, the theoretical being 11.8 per cent, and samples of sericite, kaolinite, zeolite, and white bentonite, all ground to pass a 100-mesh sieve, were treated according to the following procedure: An aqueous solution of 10 mgm. of K_2O as the chloride was added to a 1 gm. sample of the mineral, and the mixture was placed on a hot plate at 70°C. and alternately wetted and dried 20 times. The soil was then dispersed in normal neutral ammonium acetate for 15 minutes with a mechanical stirrer, after which it was recovered from the ammonium acetate by centrifuging, and the soluble potash was removed by six to eight more washings with ammonium acetate. The potassium in the filtrate was then determined by the sodium cobaltinitrite method as described by Volk and Truog (3). The amount of potash fixed was calculated by subtracting the quantity recovered with the ammonium acetate from the quantity originally added to the sample. This same procedure was used for all fixation studies reported in this paper. The results, presented in table 1, show that the minerals fixed from 0 to 1,700 p.p.m. of K_2O, which is about the range of fixation found for soils. Sericite did not fix any potash, and kaolinite fixed 450 p.p.m. One sample of white bentonite was of particular interest because it fixed 8,850 p.p.m. of potash. Further studies made on this bentonite are reported in a later part of this paper.

The question arose as to the stage of soil development in which the potash fixing material was being formed. In an attempt to answer this question, three samples, a naturally decomposed basalt, a mica schist, and a granite were tested. These rock materials were obtained from freshly decomposed parent rocks which had not been in contact with the soil proper. They were free of organic matter and other materials formed in the later stages of soil development and appeared to be in the first stage of decomposition. After the material had been passed through a 40-mesh sieve, it was treated with KCl and alternately wetted and dried 20 times at 70°C. As shown in table 1, these

materials fixed more potash than did the average soil tested by Volk (2). These data indicate that material capable of fixing potash is formed as soon as decomposition of rocks begins.

Influence of various treatments of minerals on fixation of potash

Since most of the untreated minerals fixed very little potassium, it was thought that the potassium originally present in the mineral might be removed by certain treatments and subsequently replaced by fixation. To test this possibility and also others, the various treatments tried included grinding, leaching, digestion, and the addition of colloidal silica and alumina to certain minerals. According to Volk (2), the finer soil separates are the most active in fixing potassium, which suggests that the size of the mineral particles may have some influence on fixation. Samples of three minerals were ground to

TABLE 1

Potash fixation by powdered minerals and natural rock products

MINERALS AND ROCK PRODUCTS	K_2O FIXED
	p.p.m.
Muscovite no. 2	900
Muscovite no. 6	1,300
Muscovite no. 7	1,700
Muscovite no. 18, 15, and 26	None
Sericite	None
Kaolinite	450
White bentonite	8,850
Zeolite (commercial preparation)	1,020
Zeolite (laboratory preparation)	700
Basalt	604
Mica schist	810
Granite	980

different degrees of fineness, and their capacities to fix potassium were determined. Other treatments made were designed to weather the minerals in a manner that would be somewhat similar to soil processes, such as leaching or digesting with a solution of carbon dioxide in water or with 0.1 per cent solution of Na_2CO_3. A third type of treatment consisted of adding colloidal silica and alumina to such minerals as kaolinite or pyrophyllite in an attempt to form entirely new minerals containing potash.

Influence of grinding. Sericite, kaolinite, and muscovite were first ground in an agate mortar to pass a 100-mesh sieve. Samples of this 100-mesh material were then placed in a ball mill and ground for 96 hours. They were then treated with KCl and alternately wetted and dried 20 times at 70°C. The data from this experiment, presented in table 2, show that this grinding in a ball mill did not increase the capacity to fix potash.

Influence of carbon dioxide and sodium carbonate. Samples of some of the

common soil minerals (micas and feldspars) which did not fix potash, were digested in a cold water solution of carbon dioxide; other samples of these minerals were digested in either hot or cold dilute solutions of Na_2CO_3. De-

TABLE 2

Influence of grinding on the capacity of minerals to fix potash

MINERAL	K_2O FIXED	
	By 100-mesh material	By material ground four days in a ball mill
	p.p.m.	*p.p.m.*
Kaolinite	450	500
Sericite	None	None
Muscovite no. 2	900	700

TABLE 3

Potash fixation by minerals before and after treatment with solutions of Na_2CO_3 and H_2CO_3

MINERAL	TREATMENT	K_2O FIXED	
		Before treatment	After treatment
		p.p.m.	*p.p.m.*
Muscovite no. 15 M	2 gm. to 10 l. of $H_2O + CO_2$ agitated twice daily for 37 days	0	1,320
Muscovite no. 26 M	2 gm. to 10 l. of $H_2O + CO_2$ agitated twice daily for 37 days	0	0
Muscovite no. 26 M	1 gm. to 4 l. of 0.2 per cent Na_2CO_3 and digested at 70°C. for 3 months	0	190
Muscovite no. 26 M	1 gm. to 10 l. of 0.1 per cent Na_2CO_3 and digested at room temperature for 3 months	0	460
Muscovite no. 26 M	1 gm. to 5 l. of 0.1 per cent Na_2CO_3 and digested at 70°C. for 3 months	0	0
Muscovite no. 26 M	1 gm. to 1 l. of 0.1 per cent Na_2CO_3 and placed on end-over-end shaker for 16 days	0	0
Muscovite no. 27 M	Leached intermittently for 1 year with $NaHCO_3$	0	830
Sericite	2 gm. to 4 l. of 0.2 per cent Na_2CO_3 and digested at 70°C. for 3 months	0	406
	2 gm. to 10 l. of $CO_2 + H_2O$ agitated twice daily for 37 days	0	2,600
Albite	Leached intermittently for 1 year with $NaHCO_3$	0	60
Orthoclase	Leached intermittently for 1 year with $NaHCO_3$	*	0

* Not determined

tails regarding proportions of solid to liquid, and period of treatment are given in table 3. After treatment, the liquid was filtered off, and the fixing capacity of the minerals was determined. It was thought that these treatments might produce some clay-like minerals having greatly increased fixing capacity.

The data obtained in these experiments are presented in table 3. The carbonated water treatment increased the fixation by muscovite from 0 to 1,320 p.p.m. and that by sericite from 0 to 2,600 p.p.m. Treatments with Na_2CO_3 increased the fixing capacity of the minerals from 0 to 830 p.p.m. The aforementioned processes of decomposition induce a considerable fixation and, if continued for a longer period of time, might account for all that is exhibited by soils.

Influence of additions of silica and alumina. The colloidal fraction of soils consists primarily of the clay minerals, silica, alumina, and iron oxides. Since this fraction has the greatest capacity for fixing potassium, it was thought that a combination of reactions of the clay minerals with silica, alumina, and potassium might possibly result in a secondary mineral containing potassium in a difficultly available form. Further, the clay minerals have a layer lattice structure similar to that of muscovite and, theoretically, might be changed to muscovite by the addition of potassium and aluminum to the molecule. This process is thought to take place in the formation of secondary muscovite, but the conditions necessary for such a transformation are not definitely known.

TABLE 4

Influence of additions of silica and alumina on potash fixation by kaolinite and pyrophyllite

MINERAL*	SILICA ADDED	ALUMINA ADDED	K_2O FIXED
	gm.	*gm.*	*p.p.m.*
Kaolinite	0	0	450
	0.05	0.05	450
Pyrophyllite	0	0	0
	0.05	0.05	870
	0	0.05	1,250

* 1 gm. of each.

The clay minerals selected for this experiment were kaolinite and pyrophyllite, both of which have a layer lattice type of structure. It is thought that they can be changed to muscovite under certain conditions. Pauling (1) illustrates the position of layers of atoms in a molecule of pyrophyllite and shows the changes necessary to form muscovite from it, according to the following scheme:

Pyrophyllite	*Substitute 2 Al for 2 Si*	*Muscovite*
$6\ O^{--}$		$6\ O^{--}$
$4\ Si^{++++}$	$+\ Al$	$3\ Si^{++++} + Al^{+++}$
$4\ O^{--} + 2(OH)^-$		$4\ O^{--} + 2(OH)^-$
$4\ Al^{+++}$	$=$	$4\ Al^{+++}$
$4\ O^{--} + 2(OH)^-$		$4\ O^{--} + 2(OH)^-$
$4\ Si^{++++}$	$+\ Al$	$3\ Si^{++++} + Al^{+++}$
$6\ O^{--}$		$6\ O^{--}$
	$+\ 2K$	$2\ K^+$

As a result of the replacement of one-fourth of the silicon by aluminum, a compound with 6 Al and 6 Si is obtained, but at the same time two positive charges are lost, which are balanced by adding two atoms of potassium. Thus, muscovite is formed from pyrophyllite, and in the process some potassium is fixed.

Colloidal silica, alumina, and potassium chloride were added to one set of samples of pyrophyllite and kaolinite, which was then wetted and dried as described in the fixation procedure; another set was treated with colloidal alumina and potassium chloride; and a third received only potassium chloride. The results given in table 4 reveal no increased fixation by kaolinite due to additions of silica and alumina. Pyrophyllite, however, which exhibited no fixation when potash alone was added, did fix 870 and 1,250 p.p.m. under the other two treatments. The addition of the alumina alone increased fixation more than did the addition of both silica and alumina. Thus it appears that free alumina may take part in the process of potash fixation.

Discussion

In attempting to identify the substances responsible for fixation of potash in the soil, the first logical step would seem to be to ascribe fixation to one or more of the commonly occurring minerals in the colloidal fraction. Neither the primary nor the secondary minerals studied fixed large amounts of potash. The bentonite, however, fixed considerably more potash than did any of the soils studied. Replacement of atoms within the lattice of unaltered primary minerals does not readily take place, even when the material is finely ground. Many of the secondary minerals in the soil occur in great abundance in the finer clay fraction along with colloidal alumina and silica. In the presence of this alumina and silica, the secondary minerals may more readily fix potassium, as was apparently the case in some of the experiments described.

Treatment of both the secondary and the primary minerals with carbonated water and Na_2CO_3 solution resulted in a general increase in fixation. This general increase can be attributed either to a fixation in which potassium simply enters the slightly altered molecule of the minerals, or to a combination of the added potassium with some of the decomposition products resulting from the treatments. The latter explanation is partially substantiated in the experiment with pyrophyllite in which the addition of colloidal alumina greatly increased the fixation. In this case the alumina may have entered into combination with the pyrophyllite molecule. When both colloidal alumina and silica were added to the pyrophyllite, however, the fixation was decreased, indicating that the silica is not only unnecessary but actually antagonistic to this process.

INFLUENCE OF EXTRACTION WITH SODIUM CARBONATE AND HYDROCHLORIC ACID ON FIXATION

It was found by Volk (2) that the potassium-fixing capacity of a soil was reduced by leaching with 1 *N* HCl and increased by leaching with 1 *N* Na_2CO_3.

In order to investigate this further, various high-fixing materials were treated with different concentrations of HCl and with 2 per cent Na_2CO_3 and then tested for fixing power. In this investigation the colloidal separate having particles less than 0.5μ in diameter were usually studied and will hereafter be referred to as the "colloid."

Influence of sodium carbonate extraction

One-half gram samples of the colloid from each of a Chico soil and a decomposed granite were digested in 500 cc. of 2 per cent Na_2CO_3 for 8 hours on a hot plate. The treated material was removed from the sodium carbonate solution by centrifuging, washed free of sodium with ammonium acetate, and finally washed with approximately 80 per cent alcohol. Potassium chloride was then added, and the regular potash fixation procedure was carried out as previously described.

TABLE 5

Influence of extracting the colloid from Chico soil and decomposed granite with 2 per cent Na_2CO_3 on potash fixation

EXTRACTED MATERIAL	Al_2O_3 EXTRACTED	SiO_2 EXTRACTED	K_2O FIXED	
			Before treatment	After treatment
	gm.	*gm.*	*p.p.m.*	*p.p.m.*
Chico soil (a)	0.0067	0.0157	1,150	4,500
Chico soil (b)	0.0119	0.0208	1,150	3,370
Granite (a)	0.0111	0.0443	2,100	3,050
Granite (b)	0.0259	0.0410	2,100	2,205

The results of these experiments are given in table 5. The sodium carbonate treatment tripled the fixing capacity of the colloidal separate from the Chico soil and increased that of a granite fraction about one-third. The silica and alumina removed by the sodium carbonate were determined, and it was found that the amounts extracted correlated with the fixing capacity of the separate in that the fixation was higher as decreasing amounts of alumina and silica were extracted. Removal of free silica and alumina by treatment with Na_2CO_3 and HCl, as previously described, apparently is not complete, for it will be noted that the agreement between duplicates is not close. These differences, however, probably do not seriously affect general conclusions that may be drawn.

Influence of hydrochloric acid extraction

The HCl treatments were carried out on samples of Chico, Parsons, and Summit soils, on a white bentonite, and on colloids from a decomposed granite and from the Chico soil. One-half gram samples of the bentonite and of the colloids and 5-gm. samples of the soils were each treated with 500 cc. of dilute

HCl, and the suspension was digested on the hot plate for 1 hour. The material was then recovered by centrifuging and was washed free of the last traces of HCl by repeated washings with ammonium acetate, which in turn was removed with alcohol. Potassium chloride was added to the material, and fixation was carried out in the usual manner. The effects of treatments with different concentrations of HCl are given in table 6.

Treatment of the bentonite with increasing concentrations of acid caused corresponding decreases in fixation. Occasionally, pronounced differences occurred when the same concentrations of acid were used. These differences may be accounted for by the varying amounts of silica and alumina extracted by the acid. When increasing amounts of these two substances were removed,

TABLE 6

Removal of alumina and silica from bentonite, decomposed granite, and soils by dilute HCl, and relation to potash fixation

MATERIAL	CONCENTRATION OF HCl	Al_2O_3 EXTRACTED	SiO_2 EXTRACTED	K_2O FIXED	
				Before treatment	After treatment
	N			*p.p.m.*	*p.p.m.*
Bentonite	0.01	0.0006	0.0023	8,850	7,700
	0.1	0.0024	0.0084	8,850	5,300
	(a) 0.2	0.0056	0.0186	8,850	1,300
	(b) 0.2	0.0106	0.0342	8,850	0
Parsons very fine sandy loam	0.2	0.0143	0.0109	398	92
Parsons clay loam	0.2	0.0172	0.0083	665	296
Summit clay loam	0.2	0.0229	0.0189	435	148
Chico soil	0.01	0.0028	0.0024	752	720
	0.1	0.0190	0.0152	752	650
	0.2	0.0312	0.0301	752	148
Colloid from Chico soil	0.2	0.0681	0.0542	1,150	0
	0.2	0.0771	0.0644	1,150	0
Colloid from decomposed granite	0.2	0.0213	0.0144	2,100	950
	0.2	0.0240	0.0170	2,100	625

the fixation decreased. This indicates a close relationship between the free colloidal silica and alumina and the fixing capacity of the bentonite. The results from the soils and the decomposed granite exhibit a similar correlation between the amounts of silica and alumina extracted and the fixing capacity of these materials.

Discussion

The results of the Na_2CO_3 treatment of the fine clays were similar to the results of the treatment of minerals previously described. The results obtained with the fine clays are also similar to those obtained with the minerals and would lead one to believe that the ways in which the potassium may be

fixed are the same. The Na_2CO_3 may react with the minerals of the soil, forming new compounds such as sodium aluminate or sodium silicate which have the ability to combine with the potassium to form difficultly soluble potassium alumino-silicates. The results from both the minerals and the soils indicate that potash may be fixed in several different ways.

The digestion of a soil with weak HCl seems to alter the fixing material or remove it by simple solution. The differences in amounts of alumina and silica removed seem to correlate with the resulting differences in fixation and show that when greater amounts of these oxides were removed the fixation of potassium decreased. This fact means either that the free silica and alumina in the soil are involved in the process of fixation or that the HCl breaks down the fixing materials and removes the silica and alumina liberated in this process.

INFLUENCE OF COLLOIDAL ALUMINA, SILICA, PHOSPHORIC ACID, AND CALCIUM PHOSPHATE ON POTASH FIXATION

Since the results given in tables 5 and 6 indicate that free colloidal alumina and silica are related to potash fixation, this phase of the problem was studied in more detail. To 10 gm. samples of untreated soil and soil leached with 0.2 *N* HCl, known amounts of colloidal alumina and silica were added. Another set was leached with 0.0001 *N* H_3PO_4, and to another was added monocalcium phosphate. Potassium was then added as KCl, and the regular fixation procedure was followed.

Influence of colloidal silica and alumina

The results obtained by adding colloidal alumina to soils are given in table 7. Untreated soil samples to which only alumina was added showed very little change in fixing capacity. All those which had first been digested with 0.2 *N* HCl and then treated with colloidal alumina, however, showed greater fixing power than that of the samples to which no alumina had been added. The greatest increase occurred in the high fixing materials which had been digested with HCl and then treated with colloidal alumina. The percentage increases were 29 per cent for the fine sandy loam from Honduras, 34 per cent for the white bentonite, 41 per cent for the colloid from decomposed granite, and 82 per cent for the Chico soil colloid. This indicates that the alumina removed by the HCl is definitely related to the fixing power of a soil and that replacement of this alumina in a colloidal form partly restores the fixing capacity.

When the fixing capacity of a soil was lowered by acid leaching, the leachate contained a considerable amount of silica. Results showing the effect on fixation of the addition of colloidal silica to soil are given in table 8. The addition of silica decreased fixation in all unleached samples except one, but when the soils were leached with acid and then treated with silica, potash fixation increased slightly.

In view of the fact that when soils had been leached with dilute HCl, addi-

tions of alumina decidedly increased fixation and additions of silica slightly increased fixation, the combined effect of additions of alumina and silica was determined. The amounts added ranged from 0.0648 to 0.3226 gm. of colloidal silica, and from 0.0216 to 0.3130 gm. of colloidal alumina. The results are given in table 9. When both silica and alumina were added to soils not previously leached with HCl, the fixing capacity of the soils changed very little. The addition of colloidal alumina and to silica soils previously leached with HCl, however, increased the fixing capacity slightly. When

TABLE 7

Influence on potash fixation of additions of colloidal alumina to soils, bentonite, and decomposed granite, before and after treatment with acid

MATERIALS	TREATMENT WITH 0.2 *N* HCl	COLLOIDAL Al_2O_3 ADDED	K_2O FIXED	
			No Al_2O_3 added	Al_2O_3 added
		gm.	*p.p.m.*	*p.p.m.*
Parsons very fine sandy loam	Untreated	0.0327	398	375
	Treated	0.0327	92	112
Chico soil	Untreated	0.0327	514	529
	Treated	0.0327	148	184
Parsons clay loam	Treated	0.0327	296	306
Summit clay loam	Treated	0.0327	184	211
Fine sandy loam from Honduras	Untreated	0.0216	885	851
	Treated	0.0216	311	402
Hagerstown silt loam	Untreated	0.0216	322	236
	Treated	0.0216	90	110
Chico colloid	Untreated	0.0216	620	667
	Treated	0.0216	420	765
Granite (decomposed) colloid	Untreated	0.0216	2,100	1,998
	Treated	0.0216	1,380	1,950
White bentonite (unpurified)	Untreated	0.0216	6,510	6,125
	Treated	0.0216	3,635	4,850

the amounts of silica and alumina were varied, as in Hagerstown silt loam, the fixing capacity increased when the alumina was increased and was slightly depressed when the silica was increased.

Influence of additions of monocalcium phosphate and of leaching with phosphoric acid

Samples of soils and of colloidal material from Chico soil and from naturally decomposed granite were leached with 0.0001 *N* H_3PO_4 until the absorption

of phosphorus ceased. To another set of samples, monocalcium phosphate was added at a rate equivalent to 1,000 pounds per acre on a weight basis.

TABLE 8

Influence on potash fixation of addtions of colloidal silica to soils and to the colloidal separate from decomposed granite

MATERIAL	TREATMENT WITH 0.2 *N* HCl	COLLOIDAL SiO_2 ADDED	K_2O FIXED	
			No SiO_2 added	SiO_2 added
		gm.	*p.p.m.*	*p.p.m.*
I Parsons very fine sandy loam	Untreated	0.0854	398	377
	Treated	0.0854	92	105
Chico soil	Untreated	0.0854	557	546
	Treated	0.0854	148	167
II Parsons very fine sandy loam	Treated	0.0854	296	299
Summit clay loam	Treated	0.0854	184	180
Fine sandy loam from Honduras	Untreated	0.0648	885	928
Hagerstown silt loam	Untreated	0.0648	322	274
Miami silt loam	Untreated	0.0648	334	246
Decomposed granite colloid	Untreated	0.0648	2,100	1,950

TABLE 9

Influence on potash fixation of additions of both colloidal alumina and silica to soils

MATERIAL	TREATMENT WITH 0.2 *N* HCl	COLLOIDAL Al_2O_3 ADDED	COLLOIDAL SiO_2 ADDED	K_2O FIXED BEFORE AND AFTER ADDITIONS OF Al_2O_3 AND SiO_2	
				Before addition	After addition
		gm.	*gm.*	*p.p.m.*	*p.p.m.*
Miami silt loam	Untreated	0.0216	0.0648	334	325
Hagerstown silt loam	Untreated	0.0216	0.0648	322	346
Fine sandy loam from Honduras	Untreated	0.0216	0.0648	885	926
Parsons very fine sandy loam	Untreated	0.0327	0.0854	398	376
	Treated	0.0327	0.0854	92	111
Chico soil	Untreated	0.0327	0.0854	557	500
	Treated	0.0327	0.0854	148	174
Parsons clay loam	Treated	0.0327	0.0854	296	368
Summit clay loam	Treated	0.0327	0.0854	184	226
Hagerstown silt loam	Untreated	0.1560	0.3226	322	354
	Untreated	0.3130	0.1560	322	370
	Untreated	0.3130	0.3226	322	362

Potassium chloride was finally added, and the procedure to determine fixation was then carried out as previously described. The results are given in table 10.

The fixing capacity of the materials used was decidedly reduced by leaching with 0.0001 *N* H_3PO_4. Addition of monocalcium phosphate also reduced the fixing capacity but not so much as did leaching with H_3PO_4. The fixing capacity of the Chico soil and colloid was not appreciable changed by either treatment, but the fixing capacity of all the other materials used was reduced 16 to 87 per cent. By leaching the soil with 0.0001 *N* H_3PO_4 or treating with monocalcium phosphate, the free colloidal alumina is precipitated as aluminum phosphate and becomes inactive in the processes of fixation.

TABLE 10

Influence of adding $CaH_4(PO_4)_2 \cdot H_2O$ and of leaching with H_3PO_4 on potash fixation by soils and by colloids from Chico soil and from decomposed granite

MATERIAL	TREATMENT	FIXATION OF K_2O		
		Before treatment	After treatment	Reduction due to treatment
		p.p.m.	*p.p.m.*	*per cent*
Fine sandy loam from Honduras	Leached with 0.0001 *N* H_3PO_4	885	480	46
Hagerstown silt loam	Leached with 0.0001 *N* H_3PO_4	322	42	87
Chico colloid	Leached with 0.0001 *N* H_3PO_4	620	585	6
Decomposed granite colloid	Leached with 0.0001 *N* H_3PO_4	2,100	720	66
Chico colloid	$CaH_4(PO_4)_2 \cdot H_2O$ added	620	750	0
Decomposed granite colloid	$CaH_4(PO_4)_2 \cdot H_2O$ added	2,100	1,567	25
Fine sandy loam from Honduras	$CaH_4(PO_4)_2 \cdot H_2O$ added	885	745	16
Hagerstown silt loam	$CaH_4(PO_4)_2 \cdot H_2O$ added	321	123	62
Miami silt loam	$CaH_4(PO_4)_2 \cdot H_2O$ added	334	259	22
Chico soil	$CaH_4(PO_4)_2 \cdot H_2O$ added	519	519	0

Discussion

There is considerable evidence that, at least in some soils, free alumina is either closely related to potash fixation or directly involved in the process. It may be active in the form of free colloidal alumina or as colloidal aluminates and alumino-silicates. The reaction involved is not known, but it seems reasonable to believe that colloidal alumina, aluminates, and alumino-silicate are of vital importance. This is substantiated by the fact that the Na_2CO_3 treatment of the soils appreciably increased fixation. It is possible that this treatment could form aluminates which would react with the silicates and potassium to form a relatively insoluble potassium alumino-silicate. Treatment with hydrochloric acid reduced fixation and removed considerable amounts of alumina and silica. These may have been in a free colloidal form or as aluminates and silicates. Thus, by the partial decomposition and removal of these compounds, the ability of the soil to fix potash was reduced.

The addition of colloidal alumina increased fixation but did not increase it to the original capacity of the soil before digestion with dilute HCl. It is necessary to have the alumina thoroughly distributed in the soil, and this may be the reason that the fixing capacity after treatment with dilute HCl did not equal the original capacity. On treating the soil with colloidal silica, the fixing capacity was changed very little, and it is concluded that this form of silica is not directly related to fixation. When both silica and alumina were added, the increase was about the same as that produced by the addition of alumina alone. The decreased fixation resulting from treatment with the H_3PO_4 and $CaH_4(PO_4)_2 \cdot H_2O$ also indicates that colloidal alumina may be involved, since this treatment would immobilize much of the alumina. These results do not, however, indicate that certain silicates are not also involved, because they may still form one of the links in the process.

SUMMARY

The purpose of this study was to investigate the nature of potash fixation in a difficultly available form in soils. A study was made of the fixing capacity of kaolinite, white bentonite, and zeolites (artificial); of muscovite and sericite, both before and after treatments with carbonated water and dilute sodium carbonate solutions; and of albite and orthoclase, both before and after treatment with dilute sodium bicarbonate solution. The influence on fixation of adding colloidal silica and alumina to kaolinite, pyrophyllite, soils, and soil colloids, and of grinding kaolinite, sericite, and muscovite for 4 days in a ball mill was also studied. The fixing power of soils was also determined after leaching with 0.0001 *N* H_3PO_4 and after treating with monocalcium phosphate. The influence on fixation of removing colloidal silica and alumina from soils, from bentonite, and from a freshly decomposed granite, by means of dilute solutions of sodium carbonate and hydrochloric acid, was also studied. The results may be summarized as follows:

In general, samples of untreated minerals did not fix appreciable amounts of potash, although one sample of muscovite fixed 1,700 p.p.m. of K_2O, and bentonite (a rock) fixed 8,850 p.p.m.

Potash fixing capacities of muscovite and sericite were appreciably increased by treatment with carbonated water but were not significantly changed by treatment with sodium carbonate solution.

Additions of colloidal silica and alumina did not change the amount of potash fixed by kaolinite but greatly increased the amount fixed by pyrophyllite.

The grinding of kaolinite, sericite, and muscovite for 4 days in a ball mill did not change the capacity of these materials to fix potash.

Treatment of a soil and of decomposed granite with sodium carbonate solution increased the capacity of these materials to fix potash, and treatment of these materials and of a bentonite with weak hydrochloric acid decreased this capacity. As greater amounts of colloidal alumina were extracted, the capacity to fix potash decreased, and as decreasing amounts of colloidal silica along with a constant amount of colloidal alumina were removed the capacity to fix potash decreased.

The fixing capacity of soils and of decomposed granite which had been treated with dilute

HCl, increased when alumina or both alumina and silica were added but did not change appreciably when silica alone was added.

Potash fixing capacities of decomposed granite and soils, excepting that of the Chico soil, were greatly reduced by leaching with 0.0001 *N* H_3PO_4 and by addition of monocalcium phosphate.

None of the pure minerals had a fixing power approaching that of clays, and the results do not warrant ascribing the fixing power to any definite mineral. It is significant, however, that the removal of free alumina from clays decreases fixing power, and that on replacement of this alumina the fixing power is also restored. There is considerable evidence that the products formed by Na_2CO_3 treatment, and the clay minerals easily decomposed by HCl, are associated with potash fixation. It appears that several different processes may be involved in potash fixation in soils.

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A STUDY OF THE CHEMICAL EQUILIBRIUM EXISTING BETWEEN SOLUBLE SALTS AND BASE-EXCHANGE COMPOUNDS[1]

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A review of the more recent literature concerned with the nature of base-exchange reactions in the soil indicates that the exchange of one cation for another is a true chemical reaction associated with soil colloids. Assuming this theory to be correct, one might logically expect base-exchange reactions to follow more or less closely the law of mass action. For example, it might be expected that the equation $\frac{(Na^+)\ (KZ)}{(K^+)\ (NaZ)} = C_1$ (where C_1 is a constant, Na^+ and K^+ are the activities of the respective ions, and KZ and NaZ are the activities of the unionized soil colloids) would represent equilibrium conditions found when a soil colloid is brought into contact with a solution containing both sodium and potassium salts. If the activity is constant for the unionized portion, the equation will take the form $\frac{(Na^+)}{(K^+)} = C_k$. It is well known that this type of equation holds for many crystalline substances such as $CaCO_3$ and $BaCO_3$. If this equation were applicable to soil colloids, it would be impossible to prepare, by treatment with a mixed salt solution, a colloid containing two cations in a ratio different from that in the original material. If, however, the activity of the unionized portion is dependent upon concentration, as is true with weak electrolytes in solution, a colloid could be prepared containing two cations in any desired ratio by adjusting their ratio in the treating solution. Kerr (16) developed equations similar to those given above and presented data to show that the activity of the undissociated colloid was not a constant. Vanselow (28) pointed out that similar conclusions could be reached by assuming that mixed crystals were formed by colloidal compounds containing more than one cation.

If the so-called solid phase of a colloidal suspension has a variable activity, it follows that if the colloid is treated with a solution containing two kinds of cations, a portion of each should enter the exchange complex. Webb, Jennings, and Peterson (30) report that soils treated with solutions containing Na^+ and Ca^{++} ions contained mainly replaceable calcium. This, however,

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[3] Temporarily withdrawn; resubmitted, January 22, 1938.

was not considered as in conflict with the theories of either Kerr or Vanselow but was explained by the replacing effect of $Ca(HCO_3)_2$ dissolved during the removal of excess soluble salts.

In the study reported herewith, investigations were made concerning chemical equilibrium relationships existing between soluble salts and base-exchange compounds. The purpose of the investigation was, first, to determine under what conditions a soil or bentonite, containing a mixture of replaceable cations in any desired ratio, could be prepared, and, second, to make theoretical interpretations of chemical reactions found to occur.

EXPERIMENTAL

The experimental work consisted of applying definite chemical treatments to various samples of soils and to bentonite and then analyzing the treated materials for replaceable bases.

The soil used was a heavy clay, in poor physical condition, collected from the Bell tract near Logan, Utah. The samples, nos. 1867, 1872, and 2784,

TABLE 1

Content of soluble salts found in soil 2784

M.e. per 100 gm. air-dry soil

ANION		CATION	
Cl	3.55	Na	6.45
SO_4	0.65	K	0.08
HCO_3	2.50	Ca	0.22
CO_3	0.00	Mg	0.35
SiO_3	0.80	Al	0.15
Total	7.50	Total	7.25

were those used by Webb, Jennings, and Peterson (30) for friability measurments. Soils 1867 and 2784 contained a small quantity of $CaCO_3$ (and possibly $MgCO_3$), and soil 1872 was practically free from $CaCO_3$. The soluble salts in soil 2784 (reported in table 1) were determined by the conventional methods of analyzing a 5 to 1 water extract. An examination of the data shows Cl^- and HCO_3^- to be the predominating anions and Na^+ the predominating cation. The bentonite[4] (no. 2788) was obtained from an extensive deposit in Box Elder County, Utah.

The methods adopted for the chemical treatments of soils and bentonite and for analysis of replaceable bases were, with the exception of minor modifications for bentonite,[5] the same as those reported (30) earlier from this laboratory. Briefly, they were as follows: Each sample[6] was divided into three

[4] A complete chemical analysis of the bentonite will be reported in a later publication.

[5] Wherever the methods of treating or analyzing bentonite call for different values from those used for soils, the values for bentonite are preceded by a B placed in parenthesis.

[6] This weight of sample was necessary for the friability measurements which were previously reported (30) and which were made in connection with the work herein reported.

70-gm. portions, and each portion was placed in a large Erlenmeyer flask with 250 cc. of normal chloride solution. The cation associated with the chloride in the treating solution was either a single base, such as sodium or calcium, or a mixture of two bases. The flasks were shaken several times

TABLE 2

Effect of washing upon replaceable sodium in soil 2784

Na, in m.e. per 100 gm. air-dry soil

NUMBER OF WASHINGS	REPLACEABLE Na
0	16.86
3	7.90
15	0.33

TABLE 3

Replaceable bases in soils given one chemical treatment with single-salt solutions (8 or 10 applications)

Bases, in m.e. per 100 gm. air-dry soil

LABORATORY NO.	REPLACEABLE BASES			
	Ca	Na	K	Absorbed NH_4
Soils treated with 1N $CaCl_2$				
2784	17.18	.....	.....	.....
2784	17.70	.....	.....	.....
1867	18.43	.....	.....	.....
1872	15.02	.....	.....	11.80
Soils treated with 1N NaCl				
2784	.....	6.90	.....	11.16
2784	10.40	6.19	.....	.....
1867	12.96	3.68	.....	12.78
1872	3.72	12.19	.....	12.78
Soils treated with 1N KCl				
2784	.....	.....	8.89	12.56
2784	.....	.....	11.23	.....
1867	7.08	.....	11.13	12.46
1872	2.71	.....	14.11	13.00

a day, and the suspension was allowed to settle overnight. The following morning the clear solution was siphoned off, and 250 cc. more solution was added. This procedure was repeated several times as recorded in connection with tables 3 to 7, inclusive.

After the required number of applications, the soil was washed five times

through a Pasteur-Chamberland filter. Before each washing the entire 70-gm. of soil was shaken with distilled water in a mechanical shaker. After

TABLE 4

Replaceable bases in soil 2784 given two chemical treatments

Bases, in m.e. per 100 gm. air-dry soil

FIRST CHEMICAL APPLIED*	SECOND CHEMICAL APPLIED	NUMBER OF APPLICATIONS, SECOND CHEMICAL	REPLACEABLE BASES		
			Ca	K	Na
1*N* $CaCl_2$	1*N* NaCl	10	12.23	.....	5.40
1*N* NaCl	1*N* $CaCl_2$	1	17.85	.....	0.30
1*N* NaCl	1*N* $CaCl_2$	1	17.85	.....	0.13
1*N* NaCl	1*N* $CaCl_2$	3	17.10	.....	0.18
1*N* NaCl	1*N* $CaCl_2$	3	16.48	.....	0.16
1*N* NaCl	1*N* $CaCl_2$	10	18.33	.....	0.43
1*N* NaCl	1*N* KCl	10	.....	12.31	0.02
1*N* KCl	1*N* NaCl	10	.....	1.80	5.12

* 8 on 10 applications.

TABLE 5

Replaceable bases in soil 2784 treated with mixed salt solutions (8 or 10 applications)

Bases, in m.e. per 100 gm. air-dry soil

CHEMICALS APPLIED	REPLACEABLE BASES			
	Ca	K	Na	Absorbed NH_4
Soils treated with NaCl and $CaCl_2$				
0.25*N* NaCl + 0.75*N* $CaCl_2$	17.34	.....	1.20	.....
0.5*N* NaCl + 0.5*N* $CaCl_2$	16.47	.....	1.58	.....
0.5*N* NaCl + 0.5*N* $CaCl_2$	15.15	.....	2.49	.....
0.75*N* NaCl + 0.25*N* $CaCl_2$	16.26	.....	1.42	.....
Soils treated with NaCl and KCl				
0.99*N* NaCl + 0.01*N* KCl	.....	2.98	3.06	.....
0.9*N* NaCl + 0.1*N* KCl	.....	7.07	1.15	.....
0.7*N* NaCl + 0.3*N* KCl	.....	10.41	0.52	.....
0.6*N* NaCl + 0.4*N* KCl	.....	9.46	0.38	.....
0.5*N* NaCl + 0.5*N* KCl	.....	9.95	1.37	.....
0.4*N* NaCl + 0.6*N* KCl	.....	10.31	0.19	.....
0.3*N* NaCl + 0.7*N* KCl	.....	10.36	0.09	.....
0.2*N* NaCl + 0.8*N* KCl	.....	10.71	0.14	.....
0.1*N* NaCl + 0.9*N* KCl	.....	10.73	0.11	11.93
0.01*N* NaCl + 0.99*N* KCl	.....	11.28	0.12	11.11

being washed, the three 70-gm. portions were mixed and allowed to dry. The water required to remove the last trace of solid material from the filter was

evaporated at 40°C., and the bulk of the solid matter was dried at room temperature. After drying, the soil was ground to pass through a 1-mm. sieve

TABLE 6

Replaceable bases in bentonite 2788 treated with salt solutions

Bases, in m.e. per 100 gm. bentonite dried at 110°C.

CHEMICALS APPLIED	NUMBER OF APPLICATIONS	REPLACEABLE BASES			
		Ca	Na	K	Absorbed NH_4
Bentonite treated with a single salt					
1*N* $CaCl_2$	10	131.21	0.00	.....	113.57
1*N* NCl	10	7.86	112.24	.....	.....
1*N* KCl	10	2.03	.....	102.33	101.85
Bentonite treated with NaCl and $CaCl_2$					
0.99*N* NaCl + 0.01*N* $CaCl_2$	6	16.47	103.65	.....	114.41
0.99*N* NaCl + 0.01*N* $CaCl_2$	43	12.84	99.61	.....	116.30
0.95*N* NaCl + 0.05*N* $CaCl_2$	9	26.64	85.82	.....	113.63
0.9*N* NaCl + 0.1*N* $CaCl_2$	6	50.87	66.25	.....	116.87
0.8*N* NaCl + 0.2*N* $CaCl_2$	6	71.73	40.09	.....	120.94
0.7*N* NaCl + 0.3*N* $CaCl_2$	6	110.71	12.15	.....	114.59
0.5*N* NaCl + 0.5*N* $CaCl_2$	6	116.76	11.67	.....	115.45
0.2*N* NaCl + 0.8*N* $CaCl_2$	6	127.65	3.45	.....	118.62
Bentonite treated with NaCl and KCl					
0.99*N* NaCl + 0.01*N* KCl	6	7.34	102.70	6.35	112.79
0.99*N* NaCl + 0.01*N* KCl	65	6.37	95.23	5.58	116.10
0.95*N* NaCl + 0.05*N* KCl	18	2.64	86.80	18.99	108.20
0.9*N* NaCl + 0.1*N* KCl	7	5.67	66.13	30.56	101.36
0.8*N* NaCl + 0.2*N* KCl	6	4.12	47.33	52.14	104.47
0.8*N* NaCl + 0.2*N* KCl	6	4.93	45.46	53.17	106.88
0.8*N* NaCl + 0.2*N* KCl	6	3.64	43.21	49.84	109.06
0.7*N* NaCl + 0.3*N* KCl	6	3.88	38.67	60.76	105.97
0.5*N* NaCl + 0.5*N* KCl	6	3.80	20.06	78.52	105.64
0.2*N* NaCl + 0.8*N* KCl	6	2.94	6.67	96.73	107.63
Bentonite treated with $CaCl_2$ and KCl					
0.8*N* KCl + 0.2*N* $CaCl_2$	6	26.10	0.00	80.78	104.98
0.7*N* KCl + 0.3*N* $CaCl_2$	6	52.51	0.00	69.72	100.14
0.5*N* KCl + 0.5*N* $CaCl_2$	6	56.45	0.00	53.95	107.63
0.3*N* KCl + 0.7*N* $CaCl_2$	6	82.72	.....	29.67	108.46
0.2*N* KCl + 0.8*N* $CaCl_2$	6	98.57	.....	19.28	110.44

and then thoroughly mixed. This entire procedure is spoken of as a "chemical treatment."

Two methods were used for the extraction of replaceable bases. In the

first method, 15-gm. samples were treated with six (B15) applications of an aqueous solution of 0.1*N* ammonium acetate. The solutions obtained were made up to a given volume, and aliquot portions were analyzed for sodium and potassium. In the second method, separate 15-gm. samples were treated with seven (B9) applications of 0.1*N* KCl in 50 per cent alcohol-water mixture. Solutions siphoned from the first five (B7) alcoholic applications were mixed and made up to a given volume, and an aliquot portion was analyzed for calcium. Solutions from the remaining two alcoholic applications were mixed and analyzed for dissolved calcium. Replaceable calcium was calculated by subtracting 5/2 (B7/2) of the amount found in the latter determination from the amount found in the former. When bentonite with no $CaCO_3$ added was analyzed for replaceable bases, the second extraction was dispensed with, and replaceable calcium was determined from the ammonium acetate extraction.

The replaceable sodium in the untreated soil 2784 (table 2) was calculated

TABLE 7

Replaceable bases in chemically treated bentonite 2788 containing 5 per cent $CaCO_3$ (six applications)

Bases, in m.e. per 100 gm. bentonite dried at 110°C.

CHEMICAL APPLIED	REPLACEABLE BASES			
	Ca	Na	K	Absorbed NH_4
1*N* NaCl	32.81	69.43	.....	105.86
1*N* KCl	15.19	.....	82.07	93.23
1*N* $CaCl_2$	118.17	0.00	.....	105.60
0.8*N* NaCl + 0.2*N* KCl	49.45	32.20	45.30	99.04
0.5*N* NaCl + 0.5*N* KCl	58.00	8.68	49.79	96.95
0.5*N* KCl + 0.5*N* $CaCl_2$	62.37	0.00	48.26	99.86

by subtracting the soluble sodium (table 1) from the total sodium removed by ammonium acetate solution. In all other cases, the replaceable bases were determined after the soluble salts had been removed by washing.

The absorbed NH_4 representing base-exchange capacity, was determined by removing the excess ammonium acetate with 95 per cent alcohol, then distilling NH_3 with dilute NaOH into standard acid, and titrating with standard base.

Special treatments of soils and of bentonite were as follows:

1. Soil 2784 was (a) left unwashed, (b) washed three times with distilled water, and (c) washed 15 times with distilled water (table 2).

2. Soils 1867, 1872, and 2784 were treated with single-salt solutions of $CaCl_2$, NaCl, and KCl (table 3).

3. Portions of soil 2784, treated with various single-salt solutions, were re-treated with other salt solutions, as indicated in table 4.

4. Portions of soil 2784 were treated with various mixed salt solutions of NaCl and $CaCl_2$ and of NaCl and KCl, as recorded in table 5.

5. Bentonite 2788 was treated with single-salt solutions and with mixtures of solutions of NaCl–$CaCl_2$, NaCl–KCl, and $CaCl_2$–KCl (table 6).

6. Bentonite 2788 was treated with 43 applications of 0.99*N* NaCl and 0.01*N* $CaCl_2$ and with 65 applications of 0.99*N* NaCl and .01*N* KCl respectively (table 6).

7. Enough commercial calcite was added to bentonite 2788 to give a 5 per cent mixture. Portions of this mixture were treated chemically[7] as recorded in table 7.

RESULTS

The results obtained from the experimental work are given briefly in the following paragraphs. Interpretations of the results are to be found under "Theoretical Discussion."

Virtually all the replaceable sodium was removed from soil 2784 by washing 15 times with distilled water (table 2). When soil 2784 was washed three times it contained 7.90 m.e. of replaceable sodium (table 2), a quantity only slightly greater than that found when this soil was treated chemically with NaCl (table 3). The unwashed soil contained 16.86 m.e. of replaceable sodium (table 2), much more than any sample of chemically treated soils (tables 3 and 4).

The replaceable bases present in a soil colloid after chemical treatment are independent of replaceable bases present before treatment. For example, soil 2784 which had been previously treated with $CaCl_2$ and later treated with NaCl (table 4) contained practically the same amount of replaceable sodium as when the original soil was treated with NaCl (table 3).

When the soil or the bentonite is brought into contact with a salt solution, all types of cations present in the solution enter into the exchange complex. The bases do not enter the exchange complex in the same ratio as that in which they occur in the respective treating solutions. The analyses of soils and of bentonite treated with mixed salt solutions show that the ease with which the bases enter is in the order Ca > K > Na (tables 5 and 6). This is in agreement with the work of Baver (1) and Jenny (10, 11).

All samples analyzed for calcium contained this base in a replaceable form. Samples of soils 1867 and 2784 (tables 3 and 5) and bentonite (table 7) containing $CaCO_3$ and treated with sodium and potassium salts, contained more replaceable calcium than did soil 1872 (table 3) and bentonite (table 6) practically free from $CaCO_3$ but given similar chemical treatments. The amount of replaceable calcium seemed to depend somewhat upon the cations with which the soil or bentonite was treated. In general, colloidal materials treated with a sodium salt contained more replaceable calcium than did the same materials treated with a potassium salt.

The results also indicate, first, that the base-exchange capacity of bentonite

[7] The authors express their appreciation to Joel E. Fletcher for assistance with this phase of the work.

was less when the predominating ion in the treating solution was potassium than when it was sodium or calcium (tables 6 and 7), and, second, that the sum of the separate replaceable bases is usually slightly higher than the total base capacity as indicated by the absorbed ammonia.

The tables showing analysis of replaceable bases may be used to determine the mixture of salts in the solution required to prepare (from the soil or bentonite used for this study) a soil or bentonite with two replaceable cations present in a given ratio. For example, were it desired to produce three separate samples of bentonite with the respective base-exchange ratios (expressed as milliequivalents) $\frac{Na}{K}$ $\frac{Na}{Ca}$ and $\frac{K}{Ca}$ equal to 1, interpolation of data in table 6 shows that the ratios of cations in the treating solutions must be 3.76, 6.69, and 1.38, respectively. If $CaCO_3$ were present along with the colloid, it would be impossible, by methods investigated in this study, to prepare base-exchange compounds with high ratios of either $\frac{Na}{Ca}$ or $\frac{K}{Ca}$. For soil 2784 the maximum ratio $\frac{Na}{Ca}$ is 0.59 (calculated from table 3). For bentonite 2788 containing $CaCO_3$ the maximum ratios $\frac{Na}{Ca}$ and $\frac{K}{Ca}$ are 2.12 and 5.40, respectively (calculated from table 7).

THEORETICAL DISCUSSION

The data given in this report may best be interpreted from the point of view of the chemical equilibrium existing between soluble salts and base-exchange compounds. Without a careful interpretation of this nature, a portion of the data may appear to be inconsistent. It is generally conceded that base-exchange reactions are primarily due to organic and inorganic colloids. There are conflicting theories, however, concerning the number of inorganic colloids present in the soil with base-exchange properties. Accumulated evidence (5, 15, 17, 27) tends to show that such compounds are restricted in number and are similar to but not necessarily identical (2) with those found in bentonite. Mattson (19, 20), however, considers that inorganic base-exchange compounds consist of a large number of alumino-silicates or iron silicates in which the base-exchange capacity increases with increasing silica-sesquoxide ratio. This relationship could also be explained by assuming, as Chucka (5) did when calculating the percentage of base-exchange material, that there are two or more colloids in the soil, only one having base-exchange properties.

The chemical equilibrium existing between base-exchange compounds and cations present in an aqueous solution may be represented by the network of chemical equations shown in figure 1. The system is sufficiently extensive to include modifications in equilibrium resulting from the solution's being in contact with the atmosphere or solid $CaCO_3$ or with both. In this network the customary symbols represent the activity of the respective ions and

molecules, and Z represents the activity of the negative colloid radical (alumino-silicate radical in case of inorganic colloids). This radical is shown with a valence of one, in agreement with the work of Vanselow (28). If the valence were 3, as Puri (23) suggests, two of the hydrogen atoms would be included with Z. The authors present their system of equations fully aware that the activity or so-called percentage ionization of colloids probably is due to the average distance between cations and colloid particles (11) and not to a sharp distinction between the molecules ionized and those unionized.

If the reactions represented by the network of equations obey the law of mass action (or some other known law), quantitative predictions may be made. For qualitative predictions, however, it is only necessary for the activity of the ions to increase with increasing concentration.

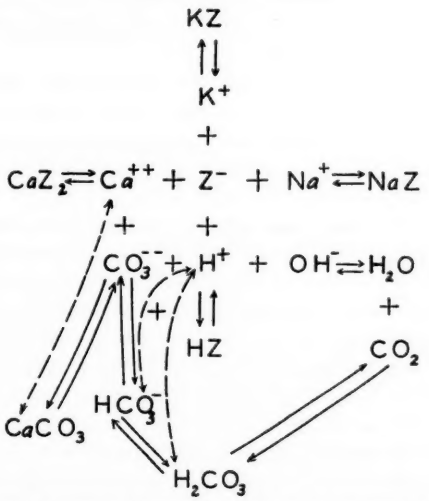

FIG. 1. A STUDY OF THE CHEMICAL EQUILIBRIUM EXISTING BETWEEN SOLUBLE SALTS AND BASE-EXCHANGE COMPOUNDS BY J. DARREL PETERSON AND D. S. JENNINGS

An examination of data in table 6 shows that, in every instance, an increase of concentration of a given cation in the treating solution resulted in an increase of this cation in the exchange complex. This indicates that in the case of bentonite the activity of the ions and molecules concerned increases with an increase of their respective concentrations.

Explanations given hereinafter are based on the following assumptions: (a) The activity is nearly constant for CO_2 when in equilibrium with the atmosphere, as well as for $CaCO_3$ and H_2O. (b) Several Z ions are combined to form a colloidal particle large enough to be retained by Pasteur-Chamberland filters and to be partially, if not wholly, retained within the soil on leaching. (c) Sufficient cations will remain with the Z ions to maintain electrical

neutrality. This is evidenced by the lower pH of colloids as compared with their ultrafiltrates (3). (d) The alumino-silicates are either weak salts or a weak acid. [Thus, Bradfield (4) calculated that if 0.001*N* $CaCl_2$ were 100 per cent ionized, Ca colloid would be 11.2 per cent dissociated.] (e) Base-exchange compounds are ionized in the following order: $NaZ > KZ > CaZ_2 > HZ$. Data in tables 3 to 7 and the lyotropic series reported in the literature (1, 11) indicate this, by the fact that the ease of replacement of the bases is in this order.

Some of the reactions reported herewith, together with a few reported in the literature, may be explained from the chemical equations represented in figure 1 and discussed hereinafter.

Soil 2784 contained more replaceable sodium (table 2) when analyzed in the presence of the soluble salts (replaceable sodium calculated as the difference between total and soluble sodium) than when this soil was given chemical treatments with sodium salts (tables 3 and 4).

The analysis of the soluble salts present (table 1) shows the predominating cation to be sodium, and a high percentage of the anions to be bicarbonate. (Probably some carbonate was present before the soluble salts were extracted for analysis.) Applying the condition just described to the equations in figure 1: The presence of HCO_3^- ion results in an increased activity of $CO_3^=$ ions and H_2CO_3. The $CO_3^=$ ions will remove Ca^{++} ions, and the hydrolysis will remove H^+ ions from HZ and H_2O. The final result will be an increase of the replaceable sodium at the expense of the replaceable calcium and hydrogen. Since all the ions and molecules are in equilibrium, the same final result could be obtained by adding NaOH to the system. This is in harmony with the relationship existing between pH and the amount of bases absorbed by the colloid and will explain the great variation of results obtained for base-exchange capacity when various methods (14, 24) are compared, as well as why the pH used during the determination must be known (6, 21).

Data presented in the report show that when soils 1867 and 2784 (tables 3, 4, and 5) or bentonite containing $CaCO_3$ (table 7) are treated with a solution originally free from Ca^{++} ions, fairly large amounts of replaceable calcium is found in the exchange-complex. This can readily be explained from the reactions represented in figure 1. Although Ca^{++} ions may be absent from the solution as added, contact with the solid material will partially dissolve $CaCO_3$, and consequently calcium will enter the exchange complex. During the washing process the concentration of all cations except calcium will decrease materially with each washing. After the soluble salts are removed, therefore, and the colloid is ready for analysis, more replaceable calcium and less replaceable sodium will be present than was there immediately following the applications of the solution. Continued washing should result in the replacement of all other cations by calcium. It was shown, by soil 2784 (table 2), that virtually all replaceable sodium was removed in 15 washings with distilled water.

A comparison of tables 6 and 7 shows that the ratios $\frac{Na}{Ca}$ and $\frac{K}{Ca}$ in the exchange complex are lower when $CaCO_3$ is present in the colloid than when absent. For example, when bentonite free from $CaCO_3$ is treated with $0.5N$ $KCl + 0.5N$ $CaCl_2$ the ratio $\frac{K}{Ca} = 0.956$ (calculated from table 6), but when bentonite containing $CaCO_3$ is given the same chemical treatment the ratio $\frac{K}{Ca} = 0.779$ (calculated from table 7). The low ratio found in the presence of $CaCO_3$ is due to the removal of sodium and potassium from the exchange complex by the calcium dissolved during the washing process. Since sodium colloid ionizes more readily than potassium colloid, the dissolved calcium will remove proportionately more sodium than potassium. For a similar reason the ratio $\frac{Na}{K}$ will be abnormally low in colloids containing $CaCO_3$.

If excess soluble salts are removed from the equilibrium system (fig. 1) by leaching or washing, the bases will be removed much more rapidly than the hydrogen ions, since the latter are partially replaced from H_2O, H_2CO_3, and HCO_3^- ions with an increase of OH^-, HCO_3^-, and CO_3^{--} ions. (If the system is not in equilibrium with the atmosphere a greater proportion of hydroxide will be formed, which may at some future time be neutralized by CO_2.) The removal of the bases will result in an increased activity of Z^- (the colloid anions). This increased activity combined with increased ratio of hydrogen to base will cause more HZ (unionized hydrogen colloid) to form, and the formation of hydroxide and carbonate will be much more pronounced. From this, one would expect not only the formation of Na_2CO_3 when sodium soils are leached (7, 8), but, to a lesser degree, the formation of $CaCO_3$ when calcium soils are leached. This may account (partly, at least) for the large quantity of Ca in Ca^+-treated bentonite, reported as replaceable in table 6.

Any other sparingly soluble calcium compounds (such as calcium silicates) would behave similarly to $CaCO_3$ in that they would furnish Ca^{++} ions to the solutions added. It may be that such compounds are responsible for the replaceable calcium found in bentonite treated with sodium and potassium salts (table 6). Increasing the number of applications of salt solutions in the treatment of bentonite reduced the quantity of replaceable bases present (table 6). The NH_4^+ ion absorbed, however, was slightly increased by these extra applications (table 6). This may indicate the removal of slightly soluble compounds. The presence of Ca^{++} ions from any source would interfere with the entrance of NH_4^+ ions into the exchange complex during the application of NH_4Ac. This condition would result in the absorbed NH_4^+ being lower than the sum of the replaceable cations, as was found to be the case with a large percentage of soil and bentonite samples analyzed.

If $CaCO_3$ is added to acid soils, neutralization of the acid releases Ca^{++} ions. Since CaZ_2 is more highly ionized than HZ, the activity of Z ions is increased,

resulting in an increase of NaZ and KZ, i.e., a decrease of Na^+ and K^+ ions. MacIntire, Shaw, and Young (18) report that the addition of $CaCO_3$ to the soil decreased the K^+ ions removed by leaching with rain water, and they considered this to be in conflict with the chemical theories of base exchange. Jenny and Shade (12) believe these results to be due to increased growth of bacteria. A detailed study of figure 1 indicates that $CaCO_3$ added to acid soils, should, according to the chemical theory advanced, decrease the K^+ ions removed. This is also shown in a publication by Peach and Bradfield (22).

These equations also furnish an explanation of why all the CO_3 cannot be recovered by simple leaching when Na_2CO_3 is added to certain soils. The Ca^{++} ions replaced by Na^+ ions will be precipitated as $CaCO_3$, lowering the CO_3^{--} ion concentration. Kelly and Brown (13) found that when Na_2CO_3 was added to Na-saturated soils, all the CO_3^{--} could be recovered.

If we assume that the degree of dispersion of colloids increases with increasing activity of Z^- ions, many phenomena associated with dispersion may be explained from figure 1. It becomes obvious that the degree of dispersion of the colloids, NaZ, KZ, and CaZ_2, will be in the same order as that of the ease in which the respective bases may be replaced. The addition of excess cations to a colloidal suspension would result in flocculation, the larger excess being required to flocculate the more highly ionized colloid. Baver (1) found the excess cations required to flocculate the respective colloids to be: Ca, 17.5 per cent; K, 282 per cent; and Na, 840 per cent. Maximum dispersion of colloids would be expected to occur in a slightly basic solution. For example, if NaOH were added, the OH^- ions would neutralize HZ, forming more Z^- ions, while the Na^+ ions would tend to remove Z^- ions. With small additions of NaOH the former reaction would dominate, causing the colloid to become more dispersed. The colloid would be flocculated, however, if large quantities of NaOH were added, since under this condition the HZ would be practically all removed, and the formation of NaZ would be the dominating reaction.

Although the success of the experiments reported herein are dependent upon the fact that colloidal particles, along with sufficient cations to maintain electrical neutrality, do not pass through Pasteur-Chamberland filters, neither the data obtained nor the equations in figure 1 give any clue as to why such large particles should be similar to weak electrolytes in that the activity of the unionized portion should vary with concentration. This similarity is probably due to the crystalline form of base-exchange compounds. According to Ross and Shannon (25) and to Wherry (31), a colloid in bentonite (montmorillonite) has a platelike crystalline form with molecular thickness in one dimension. Such crystals would have an enormous surface, approaching the total of individual molecules in solution. One may expect them, therefore, to have chemical properties similar to such molecules and yet be unable to pass through fine filters.

Figure 1 offers no explanation as to why the base-exchange capacity (absorbed NH_4) is lower for K^+-treated bentonite than for Na^+-treated or

Ca^{++}-treated bentonite (tables 6 and 7). There is a possibility that this phenomenon is related to the fixation of potassium in a nonreplaceable form as reported by Volk (29). The use of nonreplaceable potassium as a plant nutrient (9) and an increase of replaceable potassium and magnesium as a result of continued grinding (16) may also be associated with the fixation of potassium. It has been reported that all the sodium and calcium in bentonite (15) and all the calcium in soil colloids (26) are replaceable, but not all the potassium and magnesium are replaceable.

If the law of mass action holds for base exchange, the following equations will hold:

$$1.\ \frac{(Na^+)(KZ)}{(K^+)(NaZ)} = C_1; \quad 2.\ \frac{(Na^+)^2(CaZ_2)}{(Ca^{++})(NaZ)^2} = C_2; \quad 3.\ \frac{(K^+)^2(CaZ_2)}{(Ca^{++})(KZ)^2} = C_3$$

TABLE 8

Equilibrium constant of mixed Na- and K-bentonite 2788

Na/K		C_1
In treating solution	In colloid	
99.00	16.17	6.12
99.00	17.07	5.80
19.00	4.57	4.16
9.00	2.16	4.17
4.00	0.908	4.41
4.00	0.855	4.68
4.00	0.867	4.61
2.33	0.636	3.66
1.00	0.255	3.92
0.25	0.069	3.62

where C_1, C_2, and C_3 are constants and the other characters are the activities of the ions and compounds symbolized. The validity of equations 2 and 3 cannot be tested from data given in this report. For example, the ratio of $\frac{CaZ_2}{KZ}$ will vary as the excess cations are removed by washing. Each washing will remove the same fraction of one cation as another, and when water is added, a change of ratios will occur in order to adjust the activities which are squared with those which are not.

The validity of equation 1, however, may be partially tested from the data in table 6. The equilibrium constant (C_1) calculated from these data is recorded in table 8. This calculation is only an approximation, however, for the replaceable Na and K were calculated as unionized, whereas in reality the replaceable bases are the sum of those ionized and unionized after all excess cations have been removed. Before washing, the colloid will be practically unionized, because of the high concentration of cations. As washing

proceeds, the Na-colloid will ionize more than the K-colloid, with a resulting greater loss of Na^+ ions in the leaching solution. This will result in too large a value for the calculated equilibrium constant, especially for colloids high in replaceable Na. Again, it must be remembered that analysis of replaceable bases in bentonite samples treated with $0.99N$ NaCl + $0.01N$ KCl (table 6) involves the difficult determination of a small quantity of potassium in the presence of large quantities of sodium. A minute quantity of sodium weighed as potassium would account for the high value of C_1 found for these samples. Data in table 8 indicate that the law of mass action, if not rigorous, is a close approximation for base-exchange reactions.

SUMMARY

Replaceable bases were determined for soils washed with distilled water and for soils and bentonite (with and without $CaCO_3$) treated with various salt solutions. From the data obtained, the following conclusions are drawn:

Calcareous soils, when in contact with $NaHCO_3$, contain more replaceable sodium than when the soluble salts have been removed.

All replaceable sodium in calcareous soils may be replaced by continued leaching with distilled water.

When a soil or bentonite is brought into contact with a salt solution, all types of cations present in the solution enter into the exchange complex.

The bases present in the solid phase, after the chemical treatment is complete, are independent of the nature of the replaceable bases present before the treatment.

Replaceable calcium was present in soils and bentonite treated with salts of sodium and potassium. This was especially true when $CaCO_3$ was present.

The base-exchange capacity for bentonite was less when treated with potassium than when treated with sodium or calcium.

The sum of the separate replaceable bases is usually higher than the total base capacity as indicated by the absorbed ammonia.

Data presented in tables may be used to determine the mixture of salts in the solution required to prepare a soil or bentonite containing two replaceable cations present in a given ratio.

In the interpretation of many phenomena associated with base exchange, including some phenomena reported in the literature, use is made of a network of chemical equations sufficiently extensive to include equilibrium in the presence of calcium carbonate and also in contact with the atmosphere.

The following data are interpreted in the light of the chemical equations: the presence of large quantities of replaceable sodium when sodium bicarbonate or carbonate is present in the soil; the removal of replaceable sodium when calcareous soils are leached with distilled water; the presence of replaceable calcium when soils or bentonite containing slightly soluble calcium compounds is treated with sodium and potassium salts; the low value of base capacity, determined by the ammonium acetate method, as compared to the sum of the replaceable bases; a decrease of Na and K ions when $CaCO_3$ is added to acid

soils; the inability to recover all soluble carbonate added to soils; relationships existing between dispersion of colloids and chemical properties of colloids.

Phenomena reported which cannot be explained from the network of equations but which may be related to certain phenomena reported in the literature are as follows: similarity of the chemical properties exhibited by colloids and weak electrolytes and lower base-exchange capacity of potassium-treated bentonite.

Data presented indicate that the law of mass action, if not rigorous, is a close approximation for base-exchange reactions.

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THE PREPARATION, COMPOSITION, AND CHEMICAL BEHAVIOR OF THE COMPLEX SILICATES OF MAGNESIUM, CALCIUM, STRONTIUM, AND BARIUM[1]

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INTRODUCTION AND HISTORICAL

In the paper by Joffe, Kardos, and Mattson (17) on the exchange properties of artificially prepared Mg-silicate, a review was presented of the most important contributions on the subject of the rôle and behavior of Mg in the soil. The anomalous behavior of the Mg ion in the exchange complex was discussed in the light of the theories of Mattson (25, 27), Wiegner and Jenny (35), and Jenny (15). After the publication of the paper, a contribution by Wiegner (34) on the subject appeared. The distinguished investigator reported that Mg was more easily released from permutite and soil colloidal material than were the alkaline earths Ca, Ba, and Sr and that the order of release, in general, followed the lyotropic series. He suggested that the results obtained previously which gave the "anomalous" order were due to failure to prepare the various base-saturated materials in the same manner.

Other workers in the field of soil colloidal chemistry have reported conflicting data not only on the behavior of Mg, Ca, and Ba in base exchange and electrophoretic phenomena, but also on pH values for soil colloids saturated with the various alkaline earth cations.

In data reported by Anderson (1) for colloids of various SiO_2/R_2O_3 ratios which were saturated with Ca or Mg, no relation is discernible between the SiO_2/R_2O_3 ratio and the pH values of the respective Ca- and Mg-saturated material. Colloids with high ratios showed greater variations. The Fallon soil colloid with a SiO_2/R_2O_3 ratio of 3.62 gave identical pH values with both Ca and Mg; the Sharkey ($SiO_2/R_2O_3 = 3.11$) saturated with Ca had a higher pH (7.1) than that of the colloid saturated with Mg (pH 6.3); and the Marshall ($SiO_2/R_2O_3 = 2.73$) saturated with Ca had a lower pH (6.3) than that of the colloid saturated with Mg (pH 6.6). Ca-saturated colloids were indicated as having greater migration velocities than those of Mg-saturated colloids.

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Baver (3) working with Putnam silt loam clay found the Ca-saturated clay to have a lower pH and a lower migration velocity than those of the Mg-saturated clay.

Virtually all the data, probably the most recent of which have been presented by Gieseking and Jenny (9), are in agreement that the order of displacing power of the ions is $Mg<Ca<Ba$, which is in agreement with the order of hydration of these ions.

In view of the conflicting character of the available data, it was thought desirable to investigate the nature of the compounds of the alkaline earths with silica from the standpoint of stability and ionic affinity with careful control of the pH of the reacting medium. Most of the previous investigations of interaction of the alkaline earth cations with silicates were confined to fairly concentrated solutions, little attention being given to the absolute value of the hydrogen-ion concentration. Most of the products obtained were variable in composition and had the character of colloids. The aims of the investigators were directed usually toward an elucidation of the chemical processes involved in the setting of cements and mortars and in the formation and transformation of various silicates in purely geologic phenomena. Pre-eminent among these investigations are those of Lemberg, from 1870 to 1888. Lemberg prepared (20) artificial K-, Ca-, and Al-silicates, which upon treatment with $MgSO_4$ on a steam bath formed silicates that were much less soluble in H_2O than the untreated compounds. He also (22) transformed certain silicate minerals, by digestion in $MgCl_2$ solution, from predominantly CaO-bearing to MgO-bearing. Treatment of the Mg-converted mineral with $CaCl_2$ solutions failed to effect more than a 50 per cent reconversion to the Ca-bearing mineral, and in general a longer time and a more concentrated solution were required to effect an equal reconversion. The transformation of the Ca form to the Mg form was accompanied by a taking up of H_2O; it indicates that the greater hydration of the Mg^{++} ion is not inconcomitant with its greater stability and lower solubility.

Jordis and Kanter (18) working with Ba, Ca, and Sr chlorides and hydroxides obtained, with sodium silicate solutions and silicic acid sols respectively, precipitates which in general corresponded with the metasilicate formula $MO \cdot SiO_3 \cdot H_2O$.

Heldt (14) using the chloride of Ca and the sulfate of Mg with water glass ($6SiO_3 \cdot 3K_2O$) and $3Na_2O \cdot 2SiO_3$ obtained precipitates of variable appearance and composition. The precipitates of the Ca-systems varied in composition from $CaO \cdot 2SiO_3 \cdot 2H_2O$ to $5CaO \cdot 2SiO_3 \cdot 5H_2O$, and the precipitate of the Mg-system was a single compound, $MgO \cdot 2SiO_2 \cdot 2H_2O$.

The Mg-silicate studies mentioned (17) prompted a study of the other alkaline earths. It was felt that any light shed by any one of the alkaline earth cations might help in interpreting the behavior of the Mg ion.

The present work was carried on with a view of utilizing some of the more recent concepts of the colloidal micelle in interpreting the problem of the behavior of the alkaline earth cations in silicates.

EXPERIMENTAL

Part I

The first phase of the work was the study of the composition and chemical behavior of alkaline earth silicates prepared by the interaction of the chlorides of the alkaline earth metals with $Na_2SiO_3 \cdot 9H_2O$ in aqueous solutions. The method of procedure was similar to that used by Mattson (24) in preparing his isoelectric precipitates. The pH of the mixtures was varied by adding HCl to the solution of the alkaline earth salt before mixing.

The final volume in each mixture was 75 cc. The reacting concentrations were 0.005 *M* with reference to the $SiO_3^=$ ion and 0.0025 *M*, 0.005 *M*, and 0.010 *M* with reference to the alkaline earths in the A, B, and C groups. Series 1 received 0.300 m.e. of HCl, and each successive series received 0.05 m.e. more than its predecessor. The silicate and the alkaline earth chloride plus HCl were measured into separate test tubes, and the required distilled water to make up to volume was added to the alkaline earth chloride-HCl solution. The latter was then rapidly poured into the silicate solution and mixed quickly by pouring back and forth into the test tubes four times, ending with the mixture in the tube which originally contained the sodium silicate. The tubes were immediately stoppered, and the time was noted. The degree of flocculation, the relative volumes of the settled flocs, and the appearance of the precipitates were noted after 1 hour and after 18 hours. At the end of 18 hours the test tubes were shaken, and cataphoretic (with the Mattson cell) and pH determinations were made. The precipitates were then centrifuged and washed by decantation three times with 25-cc. portions of freshly boiled and cooled H_2O. The precipitates were then analyzed by the standard methods for the alkaline earth cation and silica. The data and observations are shown in table 1 and in figures 1, 2, and 3. In duplicate preparations, the average deviation in pH was 0.03 pH unit with the maximum 0.1 pH unit, and in cataphoretic measurements the average deviation was 6 per cent with a maximum of 13 per cent.

At no time was the solubility product of $Mg(OH)_2$ [$10^{-10.93}$ as shown by Gjuldback (10)] exceeded, thus excluding the possibility of precipitation of $Mg(OH)_2$.

Ba and Sr gave no precipitates with the concentrations of Na_2SiO_3 and alkaline earth chloride employed, although the test tubes were allowed to stand for 1 week.

The Ca precipitates, with the exception of those in the first two series, consisted of large flocs with vague outlines and were observed cataphoretically only with difficulty. The Mg precipitates, on the other hand, were apparently much more dense than the Ca precipitates, inasmuch as they presented sharp outlines in the field of illumination, and the settled floc presented a more densely white appearance in the test tubes.

The stronger affinity of Mg for the silicate anion as compared with Ca

TABLE 1

Na_2SiO_3-MCl_2 system

SERIES		COMPOSITION SiO_2/MO		pH		CATAPHORESIS		COAGULATION*		
		Mg	Ca	Mg	Ca	Mg	Ca	Mg, 1 hr.	Mg, 18 hrs.	Ca, 18 hrs.
						μ/sec./v./cm.	μ/sec./v./cm.			
1	A	0.970	.....	9.80	10.6+	0.244	.....	0	+	+
	B	0.960	0.820	9.12	10.6+	0.222	0.165	xx	xx	xx
	C	0.970	1.490	8.95	10.55	0.142	0.148	xxx	xxx	xxx
2	A	1.290	.....	9.14	10.30	0.248	.....	0	+	0
	B	1.230	0.910	9.08	10.35	0.164	0.163	xx	xx	x
	C	1.320	1.480	8.80	10.35	0.170	0.114	xxx	xxx	xxx
3	A	1.050	.....	9.15	10.15	0.190		0	0	0
	B	1.350	1.476	9.00	10.30	0.143		xx	xx	x
	C	1.280	1.635	8.85	10.30	0.098		xxx		xxx
4	A	3.000	.....	9.00	9.95	.....		0	0	0
	B	1.520	.....	8.85	10.10	0.149		xx	xx	+
	C	1.460	1.890	8.75	10.05	0.108		xxx	xxx	xxx
5	A	.....	.....	8.90	9.90	0.126		0	0	0
	B	1.295	.....	8.85	9.45	0.108		xx	xx	+
	C	1.585	3.180	8.65	9.45	0.111		xxx	xxx	0
6	A	.....	.....	8.85	9.75	0.137		0	0	0
	B	1.705	.....	8.70	9.40	0.099		x	x	0
	C	1.523	3.190	8.55	9.40	0.094		xxx	xxx	0
7	A	.....	.....	8.85	9.60	0.153		0	0	0
	B	2.246	.....	8.65	9.30	0.073		0	0	0
	C	1.955	8.510	8.50	9.30	0.088		xx	xx	0
8	A	.....	.....	8.80	9.38	0.146		0	0	0
	B	3.228	.....	8.65	9.10	.....		0	0	+
	C	2.572	16.650	8.50	9.05	0.092		x	x	0
9	A	.....	.....	8.85	9.05	0.109		0	0	0
	B	3.157	.....	8.45	8.85	0.114		0	0	+
	C	3.524	.....	8.30	8.85	0.106		0	+	+

Particles invisible in cataphoretic cell

SERIES		pH		SERIES		pH		SERIES		pH	
		Mg	Ca			Mg	Ca			Mg	Ca
10†	A	8.15	9.05	12†	A	3.15	7.55	14†	A	2.70	2.70
	B	8.00	8.25		B	3.15	3.15		B	2.75	2.65
	C	8.15	8.25		C	3.15	3.15		C	2.75	2.65
11†	A	4.25	8.60	13†	A	2.90	2.90				
	B	4.20	4.30		B	2.85	2.95				
	C	4.90	5.30		C	2.85	2.95				

*xxx, complete; xx, moderate; x, slight; +, trace. † Precipitates no longer were formed.

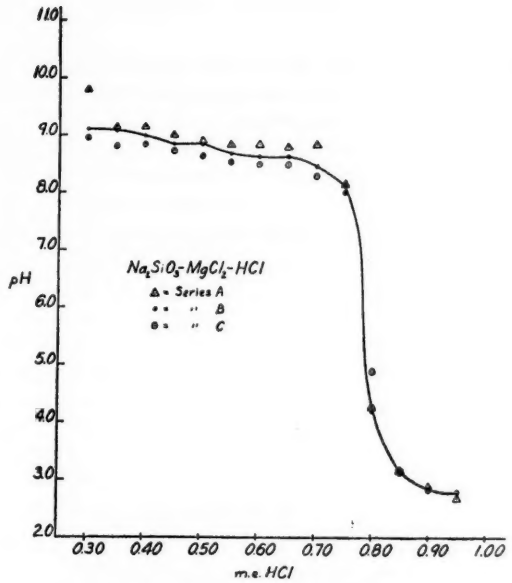

FIG. 1. THE ALKALINE Na_2SiO_3-$MgCl_2$ System
"Alkaline" in contrast to the neutralized Na_2SiO_3 system

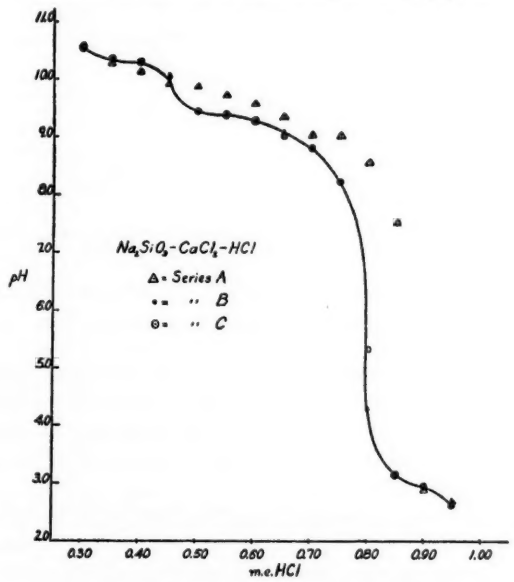

FIG. 2. THE ALKALINE Na_2SiO_3-$CaCl_2$ SYSTEM
"Alkaline" in contrast to the neutralized Na_2SiO_3 system

is evident in the pH data, wherein it is seen that throughout the series the Mg is more effective in reducing the quantity of silicate available for hydrolytic cleavage as measured by alkalinity engendered, and also in the composition data, since the SiO_2/MO ratio at any particular pH is much lower in the Mg series, the difference being accentuated at the lower values of pH.

Notwithstanding this apparently greater affinity of the Mg for the silicate anion, we see that initially the cataphoretic mobilities of the Mg precipitates are significantly greater than those of the Ca. Baver (3) also observed a higher cataphoretic mobility in Mg-saturated soil colloids than in Ca-saturated colloids.

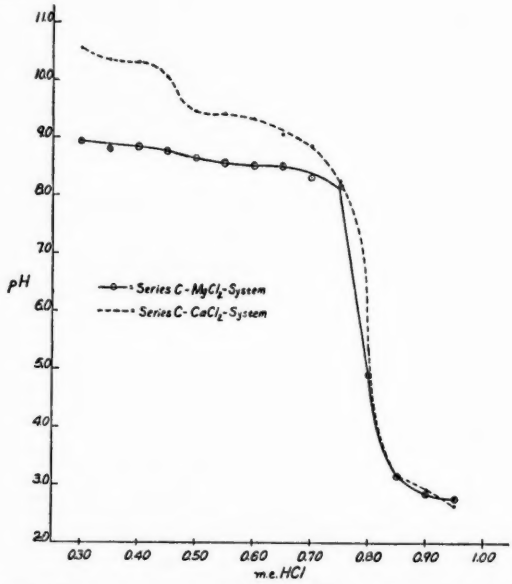

FIG. 3. SERIES C OF THE ALKALINE Na_2SiO_3-$MgCl_2$ AND Na_2SiO_3-$CaCl_2$ SYSTEMS
"Alkaline" in contrast to the neutralized Na_2SiO_3 system

The coincidence of the region of flatness in the pH curve (pH 8.5–8.65 in the Mg-systems) with the minimum in the cataphoretic mobility strongly indicates compound formation, or at least the formation of a stable phase, under the particular conditions of this experiment, notably the concentration and composition of the solutions used.

The SiO_2/MgO ratios of the B and C precipitates of series 7, in which the cataphoretic minimum occurs, are 2.246 and 1.955 respectively. Lemberg (21) reports Mg-silicates with SiO_2/MgO ratios of 2.38 and 2.28; and Heldt (14), one with a ratio of 2.0. Further investigation in this pH zone is necessary, however, before specific compound formation may be claimed.

Another point of interest to be derived from the pH curves lies in the effect of the increasing concentration of the alkaline earth chloride upon the pH values within a given series. It is apparent that the greater the concentration of the divalent cation, the greater is the depression of the pH. In each instance the greater the displacement of H^+ ions from the H_2SiO_3 which had been formed by the hydrolysis of the Na_2SiO_3, the greater was the depression in the pH value. This point will be discussed presently.

Part II

Freundlich and Cohn (8) demonstrated that silicic acid sols grow very unstable in the range of pH from 9.5 to 11.0, and Ray and Ganguly (33) described the range of pH 6 to 9 as the sensitive region, the sensitivity range narrowing as the concentration of silicate decreases.

The pH curves of the previous Ca and Mg systems indicated a very sharp drop just below pH 8. A Na_2SiO_3 solution of 0.0089 *M* concentration was adjusted, therefore, by means of 0.1 *N* HCl, to a pH value which would place the resulting silicic acid sol in the sensitive range described by Ray and Ganguly, namely, to a pH value of 7.65. This neutralized Na_2SiO_3 was then allowed to age for 4 months in order to obviate the possibility of an alteration in its reactivity during the course of the experiment, since the preparation and analysis of a single system sometimes required a period of 1 week. At the end of 4 months the pH of the neutralized Na_2SiO_3 was still 7.65, and the solution was perfectly clear and optically empty in an ultramicroscopic examination.

Mixtures were made up as in the previous experiment, a range of pH being attained by adding NaOH to the Na_2SiO_3 solution immediately before mixing. The final concentrations were the same as in Part I, the $SiO_3^=$ being 0.005 *M* and the divalent cation 0.0025 *M*, 0.005 *M*, and 0.010 *M* respectively in the A, B, and C groups. The NaOH was added in increments of 0.1 m.e. in the 50-cc. volume. Thereafter a procedure similar to that in Part I was followed. The data obtained are presented in table 2.

It is evident from table 2 that in spite of the smaller displacing power of the Mg^{++} ion as compared to the Ba^{++} ion, as reported by various investigators, we still get the most efficient coagulation with the Mg^{++} ion as measured in total milligrams of SiO_2 which was precipitated. Inasmuch as we are concerned now with a more sensitive phase, a dilute SiO_2-sol, one would normally expect those ions which are most weakly hydrated to be absorbed most strongly by the electronegative SiO_2-sol and thereby cause its flocculation. However, in coagulation phenomena with colloidal electrolytes such as SiO_2-sol, tungstic acid sols, As_2S_3 sols, and many others when brought about by the addition of salts, we have always the possibility of interaction of one of the ions of the salt with the ionic surface of the colloidal electrolyte. In some data presented by Rabinovitch and Kargin (32) for As_2S_3 and V_2O_5 sols, a process of salt formation was indicated as taking place and was governed by

the composition of the intermicellar liquid. Under such conditions, if the peptizing or coagulating electrolyte forms intermediate chemical compounds with the substance of the colloidal particle, corresponding solution pressures of the components of these compounds must exist in the surrounding liquid

TABLE 2

Neutralized Na_2SiO_3-MCl_2 system

SERIES		COMPOSITION SiO_2/MO	pH	FLOCCULATION,* 18 HRS.	SERIES		COMPOSITION SiO_2/MO	pH	FLOCCULATION,* 18 HRS.
Mg-1	A	.....	7.60	0	Mg-2	A	33.67	8.95	xxxx
	B	.....	7.60	0		B	39.31	8.90	xxxx
	C	.....	7.60	0		C	39.22	8.90	xxxx
Ca-1	A	.....	7.75	0	Ca-2	A	74.04	8.90	+
	B	.....	7.75	0		B	78.60	8.90	xxx
	C	.....	7.60	0		C	94.05	8.90	xxx
Sr-1	A	.....	7.65	0	Sr-2	A	.....	8.98	0
	B	.....	7.70	0		B	∞	8.95	x
	C	.....	7.70	0		C	∞	9.00	xx
Ba-1	A	.....	7.65	0	Ba-2	A	6.38	8.95	0
	B	.....	7.75	0		B	16.82	8.90	xx
	C	.....	7.65	0		C	11.34	8.65	xxx
Mg-3	A	11.26	9.10	xxxx	Mg-4	A	7.22	9.20	xxxx
	B	7.65	9.10	xxxx		B	Lost	9.10	xxxx
	C	9.23	9.05	xxxx		C	3.85	8.82	xxxx
Ca-3	A	∞	9.15	xxxx	Ca-4	A	∞	9.41	xxxx
	B	∞	9.15	xxxx		B	∞	9.42	xxxx
	C	∞	9.10	xxxx		C	∞	9.42	xxxx
Sr-3	A	13.83	9.18	xxxx	Sr-4	A	18.55	9.42	xxxx
	B	6.79	9.17	xxxx		B	21.70	9.42	xxxx
	C	3.97	9.10	xxxx		C	13.16	9.42	xxxx
Ba-3	A	10.95	9.10	xxxx	Ba-4	A	14.27	9.50	xxxx
	B	10.07	9.10	xxxx		B	11.93	9.43	xxxx
	C	7.55	9.04	xxxx		C	6.07	9.40	xxxx

* xxxx, complete; xxx, almost complete; xx, moderate; x, slight; +, trace.

and will be detectable to varying magnitudes depending upon the solubility of the compound formed.

With regard to the SiO_2-sol employed in the present experiment, the foregoing explanation seems a very proper one in the light of the behavior of the Mg^{++} ion in Part I with respect to its solubility when combined with the

$SiO_3^=$ ion. That the Mg ion has a greater attraction for the water molecules as indicated by its mobility is not necessarily a governing influence in determining the solubility of that ion or its salts. For example, Briggs (6) cites (from Landolt-Börnstein: "Physikal.-chem. Tabellen") the mobilities of the monovalent ion series and the solubilities of the respective chlorides as follows:

	MOBILITIES	SOLUBILITIES OF CHLORIDES AT 0°C.
Li	33.4	63.7
Na	43.5	35.7
NH_4	64.0	29.4
K	64.6	28.5
Cs	68.0	161.4

In this series we can find no relation between the degree of hydration, as indicated by mobility, and the degree of solubility, and for the divalent ion series, in which the order of hydration ordinarily accepted is Mg > Ca > Sr > Ba, we find no such regular order in the solubilities of the various salts as that listed in table 6.

At the same time it is observable that as the pH increases, the proportion of the alkaline earth cations with the exception of Ca, increases, the increase of Mg being much more pronounced than that of Ba, and that of Ba being more pronounced than that of Sr.

Berestneva and Kargin (5) found a similar relation for Ba as indicated by the following data:

pH OF SOL	AMOUNT OF Ba^{++} ABSORBED FROM 0.1 *N* SOLUTION, IN GM.-EQ./L.
7.90	0.0000
8.79	0.0438
9.85	0.0655
11.38	0.0980

The cataphoretic mobilities could not be determined upon the above flocs, since they were virtually invisible in the cataphoretic cell and also settled below the focal depth too rapidly.

The invisible character of the precipitates in the cataphoretic cell indicated that these precipitates were distinctly different from those obtained in Part I and resembled a flocculated silica sol rather than a true chemical compound of the alkaline earth cation and the neutralized Na_2SiO_3. This was found to be true when the precipitates were analyzed. The only means which could be employed to determine the migration velocity of these highly silicious flocs would have been by a moving boundary method which has been employed with silica sols. Pauli and Valko (30) using such a method obtained a value of

5.23μ/sec./volt/cm. for an electrodialyzed silica sol. Several flocs in the above series which were observable intermittently gave values between 0.2 and 0.3μ/sec. Pauli and Valko, however, used a more concentrated system, employing a KCl solution as the overlying liquid in the arms of the U-tube.

Part III

Inasmuch as we dealt with the behavior of Mg in truly ionic silicates in Part II, it was decided to carry the analysis one step further into the complex, heterogeneous system of the colloidal silicates present in the soil material.

For this purpose two soil colloidal materials, one from the B horizon of Colts Neck *loam* and the other from the B horizon of Sassafras *sandy loam*, were extracted by means of sedimentation and centrifugalization. Briefly this consisted of adding to 4 kgm. of the soil material contained in 20 l. of H_2O sufficient NH_4OH to make the suspension alkaline to litmus. After thorough mixing, the suspension was allowed to stand for a period of 18 to 24 hours, and the top half of the suspension (to a depth of about 20 cm.) was siphoned off. This process was repeated until the amount of material

TABLE 3

Composition of electrodialyzed colloids

	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	P_2O_5	C	EXCHANGE CAPACITY	SiO_2/R_2O_3
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*m.e./gm.*	
Sassafras	54.80	17.21	16.22	0.370	1.050	0.284	0.823	0.21	3.374
Colts Neck	29.00	34.53	38.03	0.095	1.056	1.160	1.309	0.32	0.837

brought into suspension became very slight. The combined siphonates were then passed through a Sharples supercentrifuge at such a rate that the effluent was practically colorless. By this means a particle size less than 2 μ was assured. These colloidal materials were then electrodialyzed in the Mattson (23) cell until a constant minimum value of approximately 0.02 m.e. of titratable bases was obtained for an interval of 24 hours. This was attained after 144 to 150 hours with a voltage of 110 to 135 and a maximum amperage of 0.10.

The electrodialyzed materials were then dried at 65°C. and ground to pass a 1-mm. sieve, after which analyses (table 3) were made by the Official A.O.A.C. (2) methods and carbon was determined by the dry combustion method. The total exchange capacity was determined by means of barium acetate at pH 7.0.

Ca-saturated and Mg-saturated materials were prepared by adding to concentrated suspensions of the H-saturated material an amount of the respective hydroxides equivalent to the total exchange capacity, then drying at 65°C., and grinding to pass at 1-mm. sieve.

The behavior of the Ca and Mg absorbed in this manner was then investigated by means of salt displacement and electrodialytic extraction.

Duplicate 1-gm. samples were leached with 250 cc. of normal ammonium acetate adjusted to pH 7.0, and the extracted Ca and Mg were determined as the oxalate and pyrophosphate respectively after removal of the NH_4Ac by evaporation to dryness on the steam bath.

Another set of duplicate 1-gm. samples were electrodialyzed, in the Mattson cell, for 16 hours at a potential of 135 volts and a current of 0.10 amperes. The dialyzates were then concentrated on a hot plate after the addition of 5 cc. of concentrated HCl, and the solution was finally evaporated to dryness on a steam bath to stabilize the silica, the presence of which was indicated in the concentrated dialyzate by its turbidity. After removal of the SiO_2 by filtration, the Ca and the Mg were determined in their respective solutions. The interference of Fe and Al was prevented by precipitating the Ca as oxalate in an acid medium and the Mg as phosphate in the presence of citrate ion.

The electrodialyzed Ca- and Mg-saturated colloids were then removed from the central chamber of the cell, transferred to a beaker, and concentrated to a volume of about 50 cc. on the steam bath. Fifty cubic centimeters of

TABLE 4

Release of cations by electrodialysis and leaching with NH_4Ac

SOIL	ELECTRODIALYSIS				NH_4Ac ALONE	
	Electrodialyzed		NH_4Ac after electrodialysis			
	Ca	Mg	Ca	Mg	Ca	Mg
	m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
Sassafras	0.170	0.037	0.069	0.083	0.228	0.194
Colts Neck	0.072	0.046	0.050	0.062	0.325	0.300

normal NH_4Ac (pH 7.0) was then added, and the materials were transferred to a funnel and then leached with an additional 200 cc. of NH_4Ac. These leachates were then analyzed for Ca and Mg. The data for this series of experiments are given in table 4.

From the data it is readily apparent that there is a distinct difference in the behavior of the Ca and Mg upon electrodialysis from a given colloidal material as well as a distinct difference in the behavior of the individual ions in the two different colloidal materials. For example, the Mg^{++} ion is removed by electrodialysis to a much smaller degree than is the Ca^{++} ion, and this difference is accentuated in the material of higher SiO_2/R_2O_3 ratio, pointing again to the rôle of the silicate portion of the molecule in stabilizing the Mg.

This relation is further demonstrated in the pH values of the respective Ca- and Mg-saturated colloidal materials. The Ca-saturated Sassafras gave a pH of 6.75 in a 1:10 suspension, and the Mg-Sassafras gave a pH of 5.90; the Ca-Colts Neck gave a pH of 6.55, and the Mg-Colts Neck, a pH of 5.60.

Another point of interest, brought out by the data in table 4, is the extreme variation in the extractability of the Ca from the Colts Neck and the Sassafras

by electrodialysis. In seeking for an explanation of this behavior we observe that the Colts Neck shows in its composition (table 3) over four times as much P_2O_5 and almost twice as much organic matter as the Sassafras. In other words, as has been repeatedly pointed out by Mattson (26), the characterization of the soil colloidal material resides not in the SiO_2/R_2O_3 ratio, but in the ratio of the activities of the acidoid and basoid fractions. This consideration, coupled with the available data indicating that the solubility of the phosphates of Ca is much less than the solubility of the phosphates of Mg and that the humates are flocculated by the Ca ion and dispersed or solubilized by the Mg ion (7), explains, in part, the difficulty encountered in electrodialyzing the Ca from the Colts Neck.

DISCUSSION AND SUMMARY

The series of experiments described indicate in some measure:

1. That the affinity of Mg^{++} ion is greater than that of Ca^{++} ion for the $SiO_3^{=}$ ion, the existence of the last ion as such having been established beyond a doubt by Harman's (12) work in the region of concentration employed.

2. That the affinity of Mg for the highly multivalent silica sol which was used in Part II can hardly be ascribed to the adsorbed OH^- and thereby to

TABLE 5

Relation of distance between lattice planes to hardness of the oxide

	Mg	Ca	Sr	Ba
Distance of particles in Å	2.10	2.40	2.57	2.77
Hardness in *moks*	6.5	4.5	3.5	3.3

the formation of insoluble $Mg(OH)_2$ in the interface, since Mukherjee (28) has indicated from purely theoretical considerations (the Debye-Hückel and Onsager theories) that the colloidal silicic acid solutions belong to the class of strong acids and are stronger than truly soluble acids of similar basicity, i.e., from the standpoint of the number of electron charges with which each particle is combined.

3. That the mutual electron affinities of Mg and O plus the ionization potentials, both of which contribute to the lattice energy and therefore to the rigidity of the bond between the associated atomic ions and the inner layer, indicate the Mg-O linkage to be stronger than the linkage of the alkaline earth cations with oxygen. The table of hardness of the oxides taken from Goldschmidt (11) indicates this relationship very markedly (table 5).

That this same Mg-O affinity is maintained and even accentuated by the presence of the silicon atom is indicated roughly by the fact that Nacken (29), investigating the heats of formation of silicates by means of the heats of solution of the oxides and the silicates in HCl–HF mixtures, found that he could not determine the heats of solution of the Mg-silicates because these compounds dissolved extremely slowly.

Kelley and Jenny (19) in a recent paper explained, on the basis of the data

of Pauling (31) on the structure of ionic crystals, the nonexchangeable and nonreplaceable Mg within the lattice as being positionally nonexchangeable but did not explain the anomalous behavior of Mg which is present in the normal exchange position. Jenny (16), in fact, has intimated that magnesium as well as beryllium fitted erratically into the base exchange equations.

That magnesium *should not* behave like Ca, Sr, and Ba is more to be expected than that it *should*. For example, among the organo-metallic compounds listed in Beilstein (4) we find only one calcium compound of the type $R-Ca-X(C_2H_5-Ca-I)$, none of strontium or barium, and hundreds of Mg compounds of various types. Especially noticeable are the large number of hydroxy-magnesium derivatives of oxy- and oxo-compounds such as:

$HO-C_6H_4-Mg-OH$,

$C_2H_5-O-C_6H_4-Mg-OH$

and

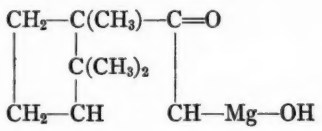

TABLE 6

Molar solubilities at 20–30°C.*

	$SO_4=$	NO_3-	CH_3COO-	$C_2O_4=$	$CO_3=$	$OH-$	$C_4H_4O_6=$	BENZOATE $C_7H_5O_2-$	SUCCINATE $C_4H_4O_4=$	CINNAMATE $C_6H_5-CH=CH-COOH$
Ba	0.00001	0.36	2.79	0.00038	0.00011	0.230	0.00096	0.118†	0.0164	0.0167†
Sr	0.0006	3.34	1.99	0.00026	0.00007	0.065	0.00800	0.167†	0.0216†	0.0310†
Ca	0.0151	7.88	2.20	0.000043	0.00013	0.022	0.00173	0.0966	0.0813	0.0063†
Mg	2.880	5.00	∞	0.0027	0.01	0.002	0.06100	0.23†	2.29†	0.027†

* From *The International Critical Tables*, vol. 4, McGraw-Hill Book Co., New York (1928).
† Molar solubilities at 15°C.

Indeed, among the organo-metallic compounds listed only those of mercury indicate any appreciable similarity to Mg with respect to types and numbers of compounds.

Moreover, it seems that the stability of the Mg in its compounds seems irrevocably related to the oxygen ion, but that the degree to which the nature and magnitude of the "force" field of this ion is modified by the atom (such as H, Si, C, P, S) to which the oxygen is attached in the hydroxide, silicate, carbonate, phosphate, or sulfate governs the solubility of the respective salts (note table 6).

A thorough analysis of the electronic configurations of the respective anions should undoubtedly reveal some paths which might lead to the complete elucidation of the behavior of the respective alkaline earth and related cations. Within the periodic table itself the anomalies of the similarity between mercury and copper, the resemblance of the behavior of magnesium to that of manganese in some of compounds, and the distinct variance of copper, silver, and

gold from the alkali elements defy, even to the present day, any complete explanation.

Undoubtedly the solution of the problem with the colloidal electrolytes lies in clarifying the picture of association, that is, the adherence of "gegenionen" (counter ions) to the micelle by virtue of electrostatic attraction, or as Hartly (13) more completely represents it, the combination of an absorption process with an atmospheric distribution, since it would not be surprising to find that the association process shows a saturation effect in solutions sufficiently concentrated. In addition, if the colloidal electrolyte exhibits a heteropolar asymmetry, the dielectric properties of the interior of the micelle, which could be previously ignored, must be taken into account because fields of force must exist within the micelle. Since this interior will in general have a lower dielectric constant than that of the solvent, the effect of the asymmetry will certainly be to make the attractive forces for a given configuration of charges greater than they would be if calculated without reference to the dielectric properties of the micelle. We should therefore expect both "atmospheric" and "association" effects to be increased and the latter more than the former.

Silicon, which is of the same periodic family as carbon, exhibits this heteropolar asymmetry to a marked extent and to an even more marked extent where the heteropolar character of the ionogen is accentuated by the presence of aluminum in the alumino-silicic acids, for example.

That the aforementioned theoretical considerations are operative in our picture of the release of the various ions upon electrodialysis might be ascertained by stripping the micelle of its atmosphere by extremely high potential fields in the cell whereby a certain limiting potential of association might be attained. To what extent this value may be correlated with the salt concentration necessary to induce a reduction in the self-potential of the exchangeable ions sufficient to make the associative effects equally low in magnitude remains to be seen.

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STUDIES IN ELECTRODIALYSIS OF SOILS: III. SPEED OF ELECTRODIALYSIS OF VARIOUS CATIONS

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It is well known that the rate of electrodialysis is different for different cations in soils (1, 4). If, however, Faraday's law is applicable, the masses that separate should be proportional to the combining weights of the ions. In other words, the quantities if reckoned in terms of milliequivalents should be the same whatever the nature of the cation in the soil. This is actually not the case; for instance, divalent ions are separated more slowly than are monovalent ions, and there are differences among ions of the same valency. These differences may be due to two causes, as follows:

1. The rate of electrodialysis may be governed by the state of aggregation of the soil particles. The greater the dispersion of the soil, the greater the rate of electrodialysis.
2. The experimental conditions in an electrodialysis cell may be such that the base once separated can recombine unless removed immediately. The rate of removal, however, may be governed by the dissociation since current can be carried only by free ions.

The object of this investigation was to see which of these two possibilities is the likely one.

INFLUENCE OF THE STATE OF AGGREGATION ON THE RATE OF ELECTRODIALYSIS

It has been shown elsewhere (2) that the state of aggregation of soil particles is governed by the nature of exchangeable bases, sodium and lithium soils being completely dispersed and other ions imparting a crumb structure of varying degrees. It was also shown that a fully dispersed soil remains as such irrespective of the changes produced in the nature of its exchangeable bases, provided it is kept in water and not allowed to dry. Thus a sodium soil which is completely dispersed can be converted into a H-soil by treatment with 0.05 *N* HCl and will remain fully dispersed in the wet condition. This H-soil can be shaken with different alkalies to produce corresponding soils, and in all cases the maximum dispersion will persist. This fact affords a simple method of varying the state of aggregation of soil without changing its exchangeable bases, and *vice versa*. The effect of state of aggregation, therefore, can be tested.

Electrodialysis was conducted in the cell with a rotating anode previously described (4). Current was maintained at 0.1 ampere with the help of a sensi-

TABLE 1

Electrodialysis of single-base soils

5-gm. portions of soil containing 25 cc. of 0.1 *N* base; current passed = 0.1 ampere

	CALCIUM SOIL		MAGNESIUM SOIL		POTASSIUM SOIL		SODIUM SOIL		AMMONIUM SOIL		BARIUM SOIL		LITHIUM SOIL		STRONTIUM SOIL	
	Recovery of base, 0.1 *N* solution	Volume of electrodialyzate	Recovery of base, 0.1 *N* solution	Volume of electrodialyzate	Recovery of base, 0.1 *N* solution	Volume of electrodialyzate	Recovery of base, 0.1 *N* solution	Volume of electrodialyzate	Recovery of base, 0.1 *N* solution	Volume of electrodialyzate	Recovery of base, 0.1 *N* solution	Volume of electrodialyzate	Recovery of base, 0.1 *N* solution	Volume of electrodialyzate	Recovery of base, 0.1 *N* solution	Volume of electrodialyzate
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
(*A*) *Soils*																
1st hour	8.30	780	3.52	806	12.19	704	19.29	577	9.73	1,270	8.50	1,366	16.85	639	7.69	1,188
2nd hour	3.53	572	1.09	572	3.12	546	3.66	343	2.70	1,018	3.15	1,117	2.60	420	2.80	920
3rd hour	2.50	626	0.95	480	2.80	450	0.84	300	1.05	800	1.70	1,150	1.50	454	2.40	1,050
4th hour	2.21	611	0.87	450	2.47	566	0.63	318	0.95	690	1.70	1,550	0.85	378	1.50	1,060
5th hour	1.84	584	0.59	384	1.99	618	0.87	380	0.70	658	1.30	1,610	0.70	415	1.80	1,185
Total	18.38	3,173	7.02	2,692	22.57	2,884	25.29	1,918	15.13	4,436	16.35	6,793	22.50	2,306	16.19	5,403
(*B*) *Soils*																
1st hour	9.14	698	2.95	615	13.54	683	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
2nd hour	3.60	637	1.58	633	3.67	431	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
3rd hour	2.40	489	1.10	518	2.20	334	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
4th hour	2.09	494	1.12	644	1.68	694	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5th hour	1.73	511	0.71	590	1.53	532	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Total	18.96	2,829	7.46	3,000	22.62	2,674	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
(*C*) *Soils*																
1st hour	10.71	350	4.53	545	17.05	321	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
2nd hour	3.06	250	1.17	436	4.05	202	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
3rd hour	1.97	308	0.86	374	1.90	187	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
4th hour	1.44	289	0.71	328	0.75	181	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5th hour	1.29	278	0.62	306	0.28	195										
Total	18.47	1,475	7.89	1,989	24.03	1,086	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
(*D*) *Soils*																
1st hour	8.24	798	3.98	600	11.15	590	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
2nd hour	3.63	480	1.59	505	4.81	245	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
3rd hour	3.14	416	1.13	444	3.20	235	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
4th hour	2.89	399	0.95	506	2.66	328	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5th hour	2.41	371	0.87	525	1.39	363	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Total	20.31	2,464	8.52	2,580	23.21	1,761	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

tive ammeter and sliding resistance. The following single-base soils were prepared from a black cotton soil of high base-exchange capacity:

(A) H-soil neutralized with equivalent amounts of different bases and kept wet.
(B) Same as (A) except that soils were dried after treatment with bases.
(C) H-soil converted into Na-soil then again into H-soil, the last neutralized with different bases and kept wet (maximum dispersion).
(D) Same as (C), except that soils were dried after treatment with bases (dispersion varying according to the nature of the cation).

A 5-gm. portion of each soil was electrodialyzed for 5 hours. Hourly estimations of the alkali removed and of the volume of the dialyzate were made, and everything else likely to influence the rate of electrodialysis was kept constant.

The results are given in table 1 and show that in every soil the differences in the rate of electrodialysis due to the nature of the cation are maintained.

TABLE 2

Electrodialysis of permutites

	SODIUM PERMUTITE		CALCIUM PERMUTITE		MAGNESIUM PERMUTITE	
	Recovery of base, 0.1 *N* solution	Volume of electrodialyzate	Recovery of base, 0.1 *N* solution	Volume of electrodialyzate	Recovery of base, 0.1 *N* solution	Volume of electrodialyzate
	cc.	*cc.*	*cc.*	*cc.*	*c.c.*	*c.c.*
1st hour	26.84	598	14.65	1,658	11.15	1,331
2nd hour	20.43	1,259	10.55	1,452	9.65	1,453
3rd hour	12.69	868	9.50	1,340	6.35	1,250
4th hour	13.63	854	5.00	1,645	4.45	1,230
5th hour	12.85	770	4.55	1,690	4.35	1,340
Total	86.44	4,349	44.25	7,785	35.95	6,604

Such differences, therefore, are definitely not due to the state of aggregation of the soil. The state of aggregation of the soil, however, is reflected in the volume of the dialyzate obtained. In spite of the large differences in the amount of the dialyzate, the quantity of the exchangeable base removed is unaffected. This fact is rather important, as it leads to the conclusion that no advantage, as regards the rate of removal of exchangeable bases, could be gained by increasing the rate of filtration in electrodialysis; in fact, it would be better to reduce the rate of filtration in order to obtain a more concentrated solution of the bases removed and to avoid analytical errors that might arise in the examination of large volumes of a solution.

The experiment was repeated with an artificial permutite used for water softening. Permutites are supposed to possess an open structure, and, therefore, the question of state of aggregation does not arise. The results, given in table 2, show that the differences due to the nature of the cations are maintained. This confirms the conclusion reached for soils. The permutite used

was in the powdered state. It was leached with $CaCl_2$ and $MgCl_2$, followed by leaching with water, to introduce the corresponding ions. It is remarkable that the rate of filtration in Na-permutite is appreciably less than that of Ca- or Mg-permutite, though the state of aggregation must be supposed to be the same. It may be that Na renders the surface of the particles gelatinous, thus causing greater resistance to the passage of water.

IONIC ACTIVITY AS THE POSSIBLE CAUSE OF VARYING RATE OF ELECTRODIALYSIS

It is difficult to find definite values for the activity coefficients of the exchangeable cations in a soil. We can, however, determine the values of some other property related to such activity and correlate them with their relative rates of electrodialysis. Such a property is conductivity, which, as is well known, is entirely due to the ionic dissociation of the solute. If the dissolved substance were not dissociated at all, the conductivity would be minimum. The conductivity of a soil suspension free from salt would be entirely due to

TABLE 3

Conductivity and rate of electrodialysis in single-base soils

EXCHANGEABLE BASE	BASE REMOVED IN 5 HOURS	CONCENTRATION OF NaCl EQUIVALENT TO CONDUCTIVITY
	m. e.	*per cent*
Li	22.50	0.023
Na	25.29	0.020
K	22.57	0.018
NH_4	15.13	0.008
Mg	7.02	0.002
Ca	18.38	0.007
Sr	16.19	0.012
Ba	16.35	0.007

the dissociation of ions on the surface, thus the measurement of conductivities of soils with different exchangeable bases would afford a comparative measure of their degree of dissociation or activity coefficient.

The soil used for electrodialysis measurements required, for neutralization, 40 m.e. of alkali per 100 gm. of soil. It can be safely assumed that up to the point of neutrality no free alkali was in the solution and the conductivity of the suspension was entirely due to the surface ionization of colloidal particles.

Four milliequivalents of alkali contained in 150 cc. of water was added to 10 gm. of soil, and after 2 hours of shaking, the conductivity of the suspension was measured in a special electrical salinometer described elsewhere (3). As this instrument gives the conductivity directly in terms of an equivalent concentration of sodium chloride, the suspension is represented as if it were a solution of NaCl having the same conductivity as the soil suspension. This method of presentation gives a direct comparison of the ionization of different cations in an easily comprehensible manner. The results are given in table 3.

The relation between conductivity and rate of electrodialysis is as good as could be expected under the circumstances and should leave no doubt as to the true cause of the differences in the rate of electrodialysis for various cations.

SUMMARY

Experimental evidence has been brought forth in support of the contention that differences in the rate of electrodialysis of different cations in soils are due to the differences in their ionic activities. Such differences would come into play only in the case of salts of insoluble acids such as alumino-silicates.

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MICROBIAL ACTIVITIES IN SOIL: III. ACTIVITY OF SPECIFIC GROUPS OF MICROBES IN DIFFERENT SOILS[1]

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The relatively resistant, dark brown or black organic substance in the soil commonly known as humus is chiefly the product of microbial activity in the transformation of undecomposed plant and animal residues. Humus is, as Waksman (13, p. 5) calls it, a natural body varying in type and highly complex in chemical composition. The dominant effect of this body and its decomposition products on specific inherent physical and chemical profile characteristics in the processes of soil formation is now generally recognized by pedologists. The chemical and physical nature of different types of soil humus is determined largely by the chemical composition of the plant and animal residues from which the humus is derived, the prevailing climatic conditions and inherent soil characteristics under which it is formed, and the character of the microflora responsible for the transformation of the undecomposed organic residues. Conversely, the kind of microflora and its activity are greatly influenced by the chemical composition of the undecomposed organic residues composing the main food supply, as well as by the climatic conditions and the inherent soil characteristics in which the microbial activities take place.

Various investigators, notably Jenny (6) and Waksman (14) have demonstrated the influence of climatic and soil characteristics on the amount and general chemical composition of soil humus. The work of Snow (8) has amply borne out the effect of these same factors on the general nature of the soil microflora, but the influence of different undecomposed plant residues on the activities of specific, dominant groups of soil microbes, and the effect of these activities on the rate and nature of transformation of various plant residues in different types of soil have not been investigated thoroughly. A study of this nature, based on two soils of similar texture but dissimilar as to parent materials and mode of development, forms the subject of this paper.

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EXPERIMENTAL PROCEDURE

Melbourne silt loam and Palouse silt loam were selected for the experiments.

Melbourne silt loam is a soil developed under the humid climate of western Washington from residual parent material derived from arenaceous shale and argillaceous sandstone. The native cover is forest vegetation consisting of a luxuriant growth of fir mixed with a few cedar, spruce, and hemlock. The surface soil is brown, and although containing a good supply of organic matter (8.06 per cent in the sample used) it is only medium in productivity when brought under cultivation.

Palouse silt loam is a typical grassland soil developed from loessial parent material under the semiarid climate of eastern Washington. The predominant native vegetation is tall bunch grass. The surface soil is very dark brown, although it has a much smaller organic matter content than the Melbourne soil (4.41 per cent in the sample used). This soil is highly productive when brought under cultivation.

Suitable quantities of the surface soils taken to a depth of 8 inches were air dried, thoroughly mixed by hand, and then passed through a 6-mesh sieve to remove coarse organic residues. Four-kilogram portions of each soil were put separately into wide-mouthed bottles to which sufficient water was added to bring the moisture content of the soils to the normal field moisture capacity. The soil water at this point is practically static with respect to capillary and gravitational movement, and the moisture content is optimum for plant growth. The bottles were allowed to stand for 4 days to insure uniform distribution of the moisture and to allow the microflora to resume normal activity. The soils were then removed from the bottles and mixed with 1 per cent finely ground, oven-dried organic residues supplemented with enough ammonium nitrate to make the total nitrogen content of the residues equivalent to that of the sweet clover hay which constituted one of them. The ammonium nitrate was added to equalize the nitrogen supply in the various residues and to provide a plentiful amount of available nitrogen for maximum microbial activity during the early stages of organic matter transformation. Thorough mixing of the soils and residues was accomplished first by hand and finally by three sievings through a 6-mesh sieve. The bottles, covered with brown paper to prevent excessive growth of algae, were connected with an absorption train for CO_2 determinations and kept for 171 days at room temperature, which varied between 20 and 26°C. The normal field moisture content of the soils was maintained within narrow limits throughout this period by addition of water when necessary. The arrangement of the bottles and the soil treatments were as follows:

SOIL TYPE	SAMPLE NO.	TREATMENT	PER CENT N	GRAMS PER KILOGRAM OF SOIL
Palouse silt loam	1	Untreated		
	2	Wheat straw + NH_4NO_3	0.562	10.000
			34.900	0.752
	3	Sweet clover hay	3.185	10.000
	4	Pine needles + NH_4NO_3	0.596	10.000
			34.900	0.742
	5	Coniferous forest duff + NH_4NO_3	0.909	10.000
			34.900	0.652
Melbourne silt loam	6	Untreated		
	7	Wheat straw + NH_4NO_3	0.562	10.000
			34.900	0.752
	8	Sweet clover hay	3.185	10.000

The experimental methods used were, in general, the same as those described in a previous publication (10). Briefly they were as follows:

Determination of microbial activity. The production of CO_2 and the numbers of bacteria, fungi, actinomyces, aerobic cellulose-decomposing bacteria, and *Azotobacter* were determined at the beginning of the experiment and at intervals of 1 to 4 days until maximum microbial counts were obtained. The intervals were then gradually increased to 10 days, 2 weeks, 3 weeks, and finally 2 months. At each sampling period the soil in each bottle was sieved twice through a 6-mesh sieve on a clean paper, and after removal of a 3-gm. sample (on the dry basis) for microbial counts, the soil was returned to the bottle and packed by gently tapping the bottle on the desk. The samples taken for microbial counts were immediately spread thinly on clean paper and allowed to dry. A 1-gm. portion of air-dried soil was taken from each sample for the preparation of a water suspension for counts of bacteria, fungi, and actinomyces determined by triplicate platings of proper dilutions of the water suspensions. Suitable quantities of soil taken from the remainder of each sample were spread evenly on silica gel plates for counts of *Azotobacter* and aerobic cellulose-decomposing bacteria.

The bacteria and actinomyces were grown on albuminate agar adjusted to pH 7.2, and the fungi, on potato extract agar adjusted to pH 3.5 by the addition of sterilized 0.4 *N* citric acid. The media are described by Fred and Waksman (3, p. 9–10). The *Azotobacter* and cellulose-decomposing bacteria were grown on silica gel plates using Winogradsky's (15) nutrient solution for the *Azotobacter*, and Waksman's (12) nutrient solution together with filter paper for the cellulose-decomposing bacteria. The plates were incubated at 28°C.

Chemical analyses. The organic carbon in the soils was determined at the beginning of the experiment by the method of Friedemann and Kendall (4), and the CO_2 evolved was measured at frequent intervals by the method of Heck (5). Suitable samples of soil were taken from the bottles as follows: A, at the beginning of the experiment; B, at 28 days; C, at 84 days; and D, at 171 days, when the experiment terminated. These samples were used for determinations of total nitrogen, nitrate nitrogen, and pH values. The total nitrogen and the nitrate nitrogen were determined by the official (1, p. 5–16) Kjeldahl and phenoldisulfonic acid methods respectively. The pH values were determined by means of the quinhydrone apparatus using suspensions of 1 part of soil in 5 parts of distilled water.

EXPERIMENTAL RESULTS

Since only wheat straw and sweet clover hay were used in studying the course of organic residue transformation in both soils, these residues will be considered first. Pine needles and coniferous forest duff, which were added to one soil only, will receive attention subsequently.

Activity of specific groups of microbes

The trend of activity of various groups of microbes in the Palouse and Melbourne soils, untreated and treated with wheat straw and sweet clover hay, is illustrated in figures 1 to 4 inclusive. As might be expected, the graphs show a considerable fluctuation in numbers of organisms in the various groups of microbes at different times. This is accounted for, in part, by variations in microbial activity and also by the limitations of the plate count methods, even though the numbers represented by the graphs are averages of triplicate plates.

A marked difference is indicated in the activity of various groups of microbes, particularly the bacteria, actinomyces, and fungi of the untreated samples of the Palouse and Melbourne soils. Although the texture of these two soils is similar, many of their other common inherent characteristics are contrasting in nature. Two of the important ones in this connection are reaction and humus content, on which data are presented in table 1. These two factors can be expected to have a marked influence upon the kind of soil microflora that will predominate. The numbers of bacteria and actinomyces in the Palouse soil were much greater than those in the Melbourne soil, whereas the numbers of fungi were much greater in the latter than in the former. It is likely that the approximately neutral reaction of Palouse silt loam favored the activity of bacteria and actinomyces, and the acid reaction of Melbourne silt loam favored the growth of fungi. This difference in soil reaction, however, is not the only factor and probably not the major factor responsible for the difference in the microflora. Closely associated with reaction are distinct inherent characteristics which definitely affect the nature of the mineral nutrient supply as well as the physical and chemical character of the soil. These characteristics probably have a greater influence on the nature of the microflora in the two soils than does the difference in reaction. In a previous publication (11) it was shown that changing the reaction of Palouse silt loam from approximate neutrality to a pH value of 5.5 by the addition of acids, or to a pH value of 8.1 by the addition of calcium carbonate, had no appreciable effect on the numbers of bacteria. The $CaCO_3$-treated samples suffered a large reduction in the numbers of fungi, but the increased acidity of the acid-treated samples, which had a pH value approximating that of the untreated Melbourne silt loam used in the present study, caused only a moderate increase in the number of fungi. The only supply of readily available energy used in the previous work was cellulose in the form of filter paper, and no comparison was made of the effect different forms of organic materials might have on the comparative activity of different groups of microbes.

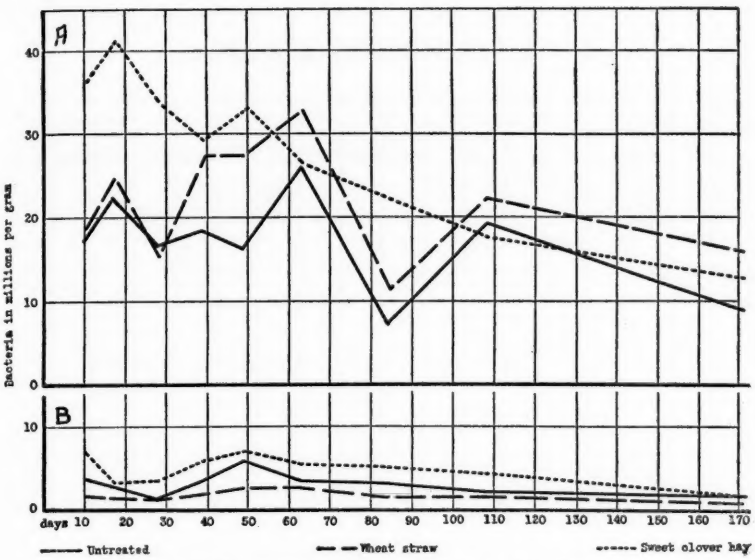
A
B
Bacteria in millions per gram
days 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170
Untreated
Wheat straw
Sweet clover hay

FIG. 1. NUMBER OF BACTERIA PER GRAM OF DRY SOIL
A, Palouse silt loam; B, Melbourne silt loam

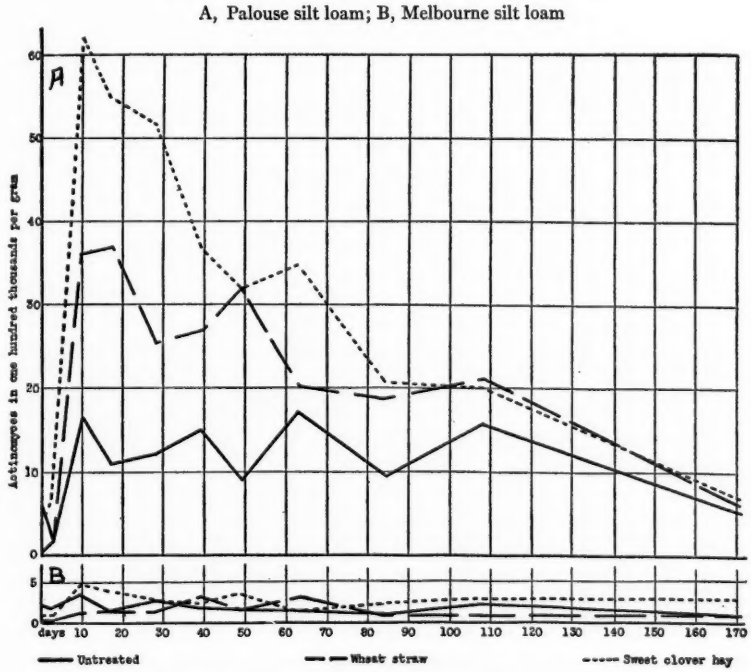
A
B
Actinomyces in one hundred thousands per gram
days 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170
Untreated
Wheat straw
Sweet clover hay

FIG. 2. NUMBER OF ACTINOMYCES PER GRAM OF DRY SOIL
A, Palouse silt loam; B, Melbourne silt loam

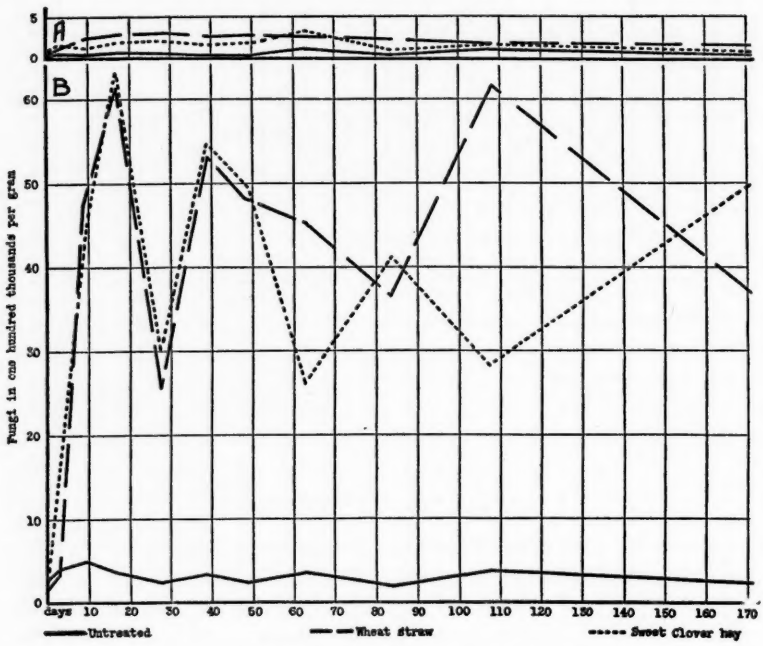
A
B
Fungi in one hundred thousands per gram
days 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170
Untreated
Wheat straw
Sweet clover hay

FIG. 3. NUMBER OF FUNGI PER GRAM OF DRY SOIL
A, Palouse silt loam; B, Melbourne silt loam

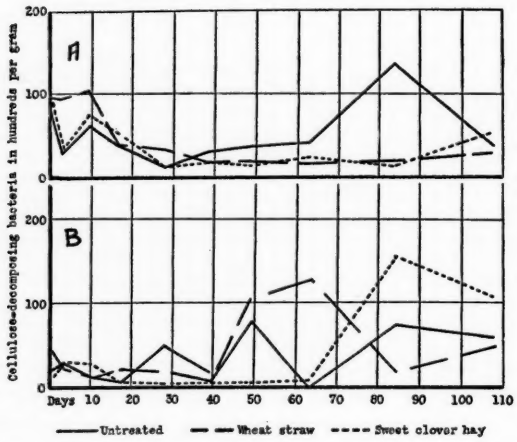

FIG. 4. NUMBER OF AEROBIC CELLULOSE-DECOMPOSING BACTERIA PER GRAM OF DRY SOIL
A, Palouse silt loam; B, Melbourne silt loam

The addition of 1 per cent wheat straw and sweet clover hay respectively to both the Palouse and the Melbourne soils caused a preponderant increase in activity of the bacteria, actinomyces, and fungi, as may be observed from figures 1 to 3. The two residues affected the activity of the same groups of microbes in different ways in the two soils, although in general the relative order in predominance of the various groups remained similar. In the Palouse soil the effect of the added residues was greatest on the activity of the bacteria and actinomyces and comparatively small on the activity of the fungi. The same residues in the Melbourne soil had by far the greatest and most persistent effect on the activity of the fungi and only a relatively small effect, particularly that of the straw, on the activity of the bacteria and actinomyces.

At the peak of activity the bacteria and actinomyces populations in the untreated Palouse soil amounted to 26,420,000 and 1,750,000 respectively per gram of soil on the dry basis, whereas in the untreated Melbourne soil the numbers were 3,890,000 and 315,000 respectively. The differences in favor of the Palouse soil are in the ratio of 1:6.8 for the bacteria and 1:5.5 for the actinomyces. The straw-treated samples had at the peak of microbial

TABLE 1

Reaction and carbon and nitrogen contents of the untreated soils

SOIL	pH	C	N	C/N
		per cent	*per cent*	
Palouse silt loam	6.73	2.569	0.196	13.0
Melbourne silt loam	5.33	4.674	0.249	18.8

activity 32,651,000 bacteria and 3,708,000 actinomyces per gram for the Palouse soil, and 2,870,000 bacteria and 278,000 actinomyces per gram for the Melbourne soil. The difference in favor of the Palouse soil is in the ratio of 1:11.4 for the bacteria, and 1:13.3 for the actinomyces. The straw stimulated both bacterial and actinomycete growth to a much greater extent in the Palouse than in the Melbourne soil. When the peak of microbial activity was reached in the samples treated with sweet clover hay, Palouse silt loam contained 41,251,000 bacteria and 6,203,000 actinomyces per gram, and the Melbourne soil contained 7,224,000 bacteria and 472,000 actinomyces per gram. The ratios in favor of the Palouse soil in these samples are 1:5.7 for the bacteria and 1:13.1 for the actinomyces, indicating that sweet clover hay stimulated the growth of bacteria about equally in the two soils, and stimulated the growth of actinomyces to a much greater extent in the Palouse soil than in the Melbourne soil. Although the predominance in numbers of bacteria and actinomyces was maintained in the Palouse soil, nevertheless these groups of organisms were affected differently by the nature of the easily available supply of energy. Their activities were considerably less in the straw-treated samples than in the samples treated with sweet clover hay.

A similar effect was evident in the behavior of the fungi which, as has already been shown, were much more numerous in the Melbourne soil than in the Palouse soil regardless of treatment. The fungus population at the peak of activity in the untreated soils was 56,700 and 486,000 per gram for the Palouse and Melbourne soils respectively or in the ratio of 1:8.5 in favor of the latter. In the straw-treated samples the maximum numbers of fungi were 288,000 and 6,195,000 per gram of the Palouse and Melbourne soils respectively, the ratio being 1:23.5 in favor of the latter. The maximum numbers in the samples treated with sweet clover hay were 314,000 and 6,300,000 per gram for the Palouse and Melbourne soils respectively, or in the ratio of 1:20 in favor of the latter. Both the straw and the sweet clover hay served as better stimulants for the growth of fungi in the Melbourne soil than in the Palouse soil, but the effect of the straw was more pronounced than that of the sweet clover hay.

Coming back to the effect of various factors on the type and activity of the microflora in the two soils employed, we see clearly that although the activity of the important groups of soil microbes concerned with the transformation of undecomposed organic residues is definitely influenced by the kind and amount of readily available carbonaceous food, the nature of the microflora is governed predominantly by the inherent soil characteristics including reaction. The soils were kept under identical conditions of temperature and moisture and received the same quantities of organic residues. As will be seen later, the total microbial activity as expressed by CO_2 evolution, NO_3 accumulation, and total numbers of microbes as determined by plate counts was not proportional to the original organic content of the soils.

The first stages of decomposition of the organic residues added to the two soils were not marked by any distinct sequence in development of the specific groups of microbes such as was observed in previous work (10) with Palouse silt loam. Unfortunately, the bacterial counts for the first and third day of the experiment were lost, but the available data indicate generally that the maximum numbers of bacteria and actinomyces occurred sometime between the tenth and seventeenth day after the residues were incorporated with the soils. The numbers in these groups remained relatively high for about 50 to 65 days when they subsided rapidly, particularly those of the bacteria in the Palouse soil. The effect of the straw treatment in the Melbourne soil was not very pronounced in this respect.

The maximum activity of the fungi was not reached generally until the seventeenth day after the experiment was started and was reached even later in some samples. Since plate counts for fungi, according to Jensen (7), are more adaptable for spores than for hyphae it is possible that maximum vegetative growth preceded the maximum numbers indicated by the plate counts; consequently, the maximum stimulation of fungal growth may have been coincident with that of the bacteria and actinomyces. The more significant feature pointed out by the data in figures 1 to 3, however, is that the stimulated

activity of the fungi as a result of the added organic residues, especially in the Melbourne soil, persisted for a much longer time than did that of the other two groups of microbes. This is an indication of the tendency toward sequential development of various groups of microbes and shows also that fungi as a group are more capable of attacking the less readily available carbonaceous substances than are the bacteria, in particular, and possibly also the actinomyces.

The trend of the activity of the aerobic cellulose-decomposing bacteria in the untreated as well as in all the treated samples of both soils is represented graphically in figures 4 and 5, where it is shown that the activity of these organisms was small regardless of soil type or treatment. The addition of organic residues to the soils had no significant effect on the activity of the aerobic cellulose-decomposing bacteria at any time during the course of the experiment which lasted 171 days, except in the soil treated with coniferous duff, where the number of these organisms began to increase about 3 months after the residue was added and was still higher, but yet relatively insignificant, at 108 days. At the end of the experiment the number, which is not indicated in figure 5 though determined, had subsided to normal.

The activity of the *Azotobacter* was even more insignificant than that of the cellulose-decomposing bacteria. No *Azotobacter* were found in the Melbourne soil regardless of treatment, and the numbers in the Palouse soil never exceeded 30 colonies per gram of soil irrespective of treatment. The data on their numbers are not included therefore, in this paper.

The trend of activity of the bacteria, actinomyces, and fungi in the samples of Palouse silt loam, untreated and treated with 1 per cent of pine needles and with 1 per cent of coniferous forest duff, is indicated in figures 5 and 6. The effect of these residues on the activity of the aforementioned groups or organisms in this soil is shown to be generally similar to the effects of straw and sweet clover hay. Some significant differences, however, in the response of individual groups of microbes to the various residues may be observed. The forest duff, which contained some partially decomposed organic material, appeared to stimulate the activity of the bacteria, actinomyces, and fungi in about the same order of magnitude as did the wheat straw, which was less effective than the sweet clover hay. The effect of pine needles on fungal growth was similar to that of sweet clover hay throughout the experiment, but its effect on bacterial activity, although similar to that of sweet clover hay during the first 50 days, persisted for about 20 days longer before subsiding. On the other hand, the effect of pine needles on the activity of actinomyces during the first 50 days was less than that of any of the other organic residues, but for a short time thereafter it reached a maximum which was greater than that of the other residues.

These differences in behavior of specific groups of microbes in separate samples of the same soil kept under identical temperature and moisture conditions but treated with different organic residues supplying similar amounts

of available nitrogen, reflect definitely the influence a particular kind of organic food supply may have on the activity of different groups of soil microbes. In the long run, any specific type of organic residue which is returned to the soil repeatedly at regular seasonal intervals during the course of soil development together with the particular temperature and moisture conditions under

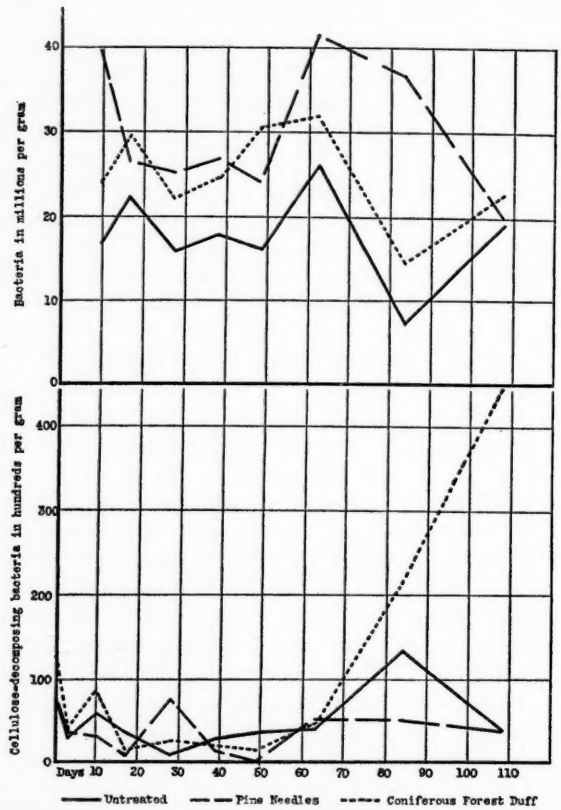

FIG. 5. NUMBER OF BACTERIA AND AEROBIC CELLULOSE-DECOMPOSING BACTERIA PER GRAM OF DRY PALOUSE SILT LOAM

which it is decomposed should have a definite effect upon the type of soil microflora that will persist. The decomposition products that will result from the transformation of this organic residue by microbial activities will determine in large measure not only the character of the soil humus formed, but also the character of the microbial habitat, which, as shown by the foregoing data, has a powerful influence on the type of soil microflora that will survive.

CO_2 evolution and nitrification in relation to microbial numbers

The approximate daily rate of CO_2 evolution and the total numbers of microbes as determined periodically by plate counts are shown graphically in figures 7 and 8 for the untreated samples of the Palouse and Melbourne soils and also for the samples treated with wheat straw and sweet clover hay. Similar data for the untreated sample of Palouse soil as well as for the samples of this soil which were treated with pine needles and coniferous forest duff are presented in figure 9.

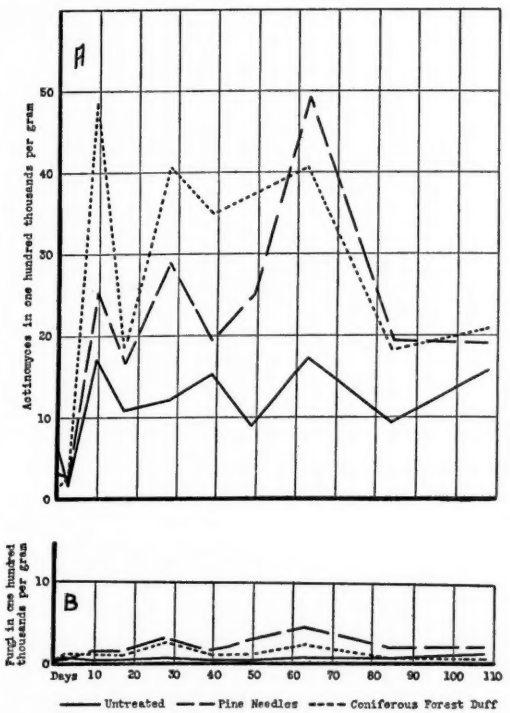

FIG. 6. NUMBER OF FUNGI AND ACTINOMYCES PER GRAM OF DRY PALOUSE SILT LOAM

The outstanding features indicated in figures 7 and 9 for those samples which received organic residues is the profuse evolution of CO_2 during the first three or four days followed by a rapid decline for the next 10 or 12 days, whereafter the rate of CO_2 evolution, although still greater than that in the untreated samples, ceased to be extraordinary. The maximum evolution of CO_2 occurred on the second day of the experiment in all the samples except the forest-duff-treated sample, which produced the largest quantities on the first day. If the rate of CO_2 evolution were a true index of microbial activity

as reflected by numbers, one could expect maximum numbers of soil microbes to coincide closely with maximum production of CO_2. A comparison of the data in figures 7 to 9 discloses that the maximum microbial development caused by the addition of organic residues to the soil, and indicated by total numbers determined by plate counts, occurred about 15 days later than the maximum CO_2 evolution. To be sure the plate count method even with the use of selective media is inadequate for the accurate determination of total

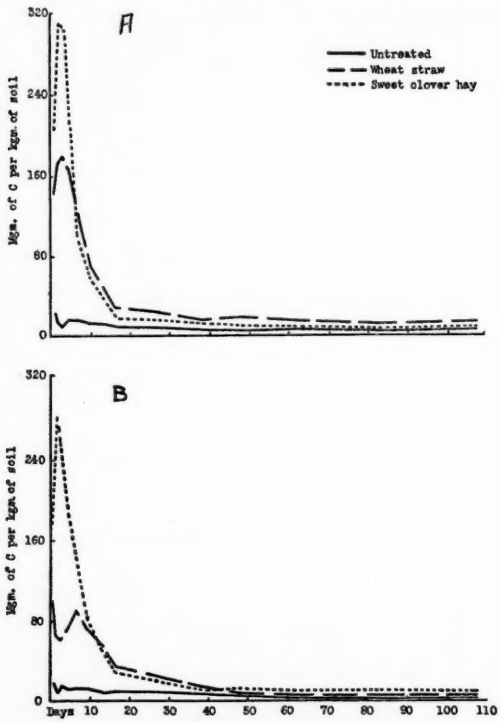

FIG. 7. DAILY CARBON DIOXIDE PRODUCTION BY SOIL
A, Palouse silt loam; B, Melbourne silt loam

numbers of individual microbes in the soil, as many of the colonies developing on the plates may represent several to many individual cells. Moreover, many cells and even species fail to grow on the nutrient media ordinarily used. Nevertheless, it is believed that those species which take the most active part in the transformation of organic matter in the soil are well represented by this method, and therefore, the results obtained from different soils should be comparable. Thus, if a maximum in numbers as obtained by plate counts is representative of maximum microbial development in the soil, the lag

between maximum CO_2 evolution and maximum numbers of organisms shown in figures 7 to 9 inclusive would indicate that the rate of CO_2 evolution is not an accurate index of microbial development. A similar lag period was noted in previous work (10). The work of Jensen (7), who studied the course of decomposition of organic residues in sand-kaolin mixtures, shows that at temperatures of 28°C. or higher similar results were produced.

It is noticed also that the first peak in microbial numbers, which in the treated soils of both the Palouse and the Melbourne series occurred about 17 days after the organic residues had been added, was followed in general by

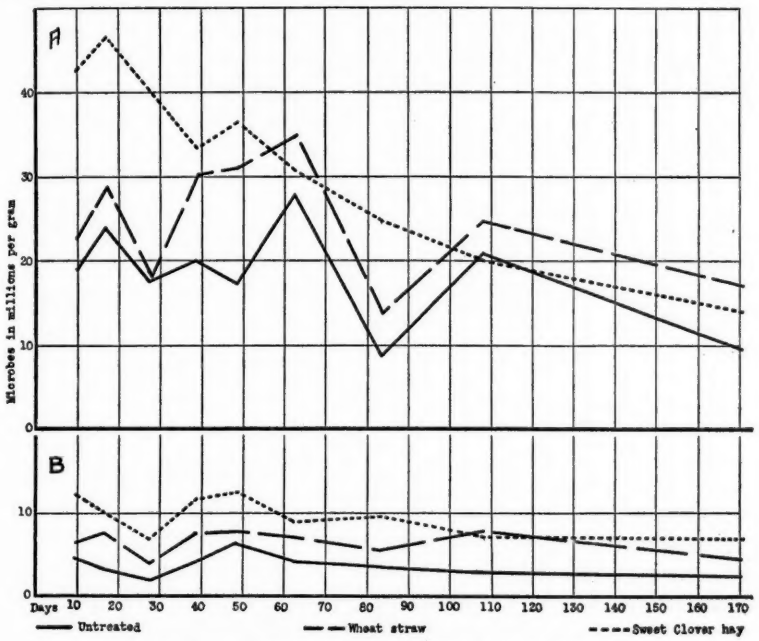

FIG. 8. TOTAL NUMBER OF MICROBES PER GRAM OF DRY SOIL
A, Palouse silt loam; B, Melbourne silt loam

a sharp decline. Later on a second peak developed at a time when the rate of CO_2 evolution was very low, showing that in none of the soils which received organic residues was the rate of CO_2 evolution a reliable index of the numbers of active microbes. Lags between maximum CO_2 production and maximum microbial numbers in soils, for which various explanations have been offered, have been observed by a number of investigators. Fischer (2) assumed that after a period of stimulated activity associated with increased CO_2 production the bacteria go into a more or less inactive resting stage in which they have a low respiratory power but are capable of germination and growth on plates.

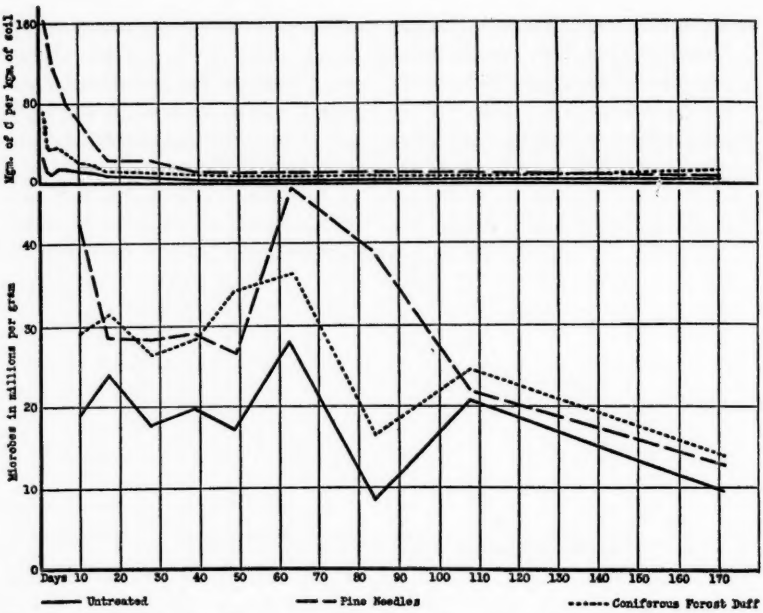

FIG. 9. CARBON DIOXIDE PRODUCTION AND TOTAL NUMBER OF MICROBES PER GRAM OF DRY PALOUSE SILT LOAM

TABLE 2

Nitrate nitrogen, pH values, and CO_2 production in the variously treated soils at the periods indicated

SOIL AND TREATMENT	BEGINNING		29 DAYS		85 DAYS		171 DAYS		
	pH	NO_3–N	pH	NO_3–N	pH	NO_3–N	pH	NO_3–N	C as CO_2
		p.p.m.		*p.p.m.*		*p.p.m.*		*p.p.m.*	*gm./kgm.*
Palouse silt loam:									
Untreated	6.73	26.8	6.46	53.3	6.29	78.3	6.24	104.2	1.32
Wheat straw	6.60	113.0	6.22	182.4	6.10	231.2	6.00	236.4	3.31
Sweet clover hay	6.65	26.2	6.47	152.8	6.30	228.6	6.12	264.4	3.30
Pine needles	6.39	104.0	6.34	157.7	6.18	195.2	6.08	214.3	2.76
Coniferous duff	6.47	110.4	6.05	209.6	5.95	244.0	5.89	308.5	1.92
Melbourne silt loam:									
Untreated	5.33	57.0	5.25	19.9	5.06	49.8	4.99	60.1	0.80
Wheat straw	4.95	104.8	4.86	134.8	4.65	187.5	4.48	230.8	2.18
Sweet clover hay	5.26	11.4	4.88	113.9	4.70	164.4	4.59	208.4	3.02

This may account for a brief lag period between maximum CO_2 production and maximum numbers and for the persistence of large numbers for some time after CO_2 production has fallen to a low point, but it does not explain a prolonged lag period of 2 weeks or more unless young cells do not develop on plates or the reproduction is much slower than is generally accepted. It seems that either much more energy is required by the soil microflora during the first stages of renewed activity or a large part of the energy released by the decomposition of readily decomposable carbonaceous substances is dissipated.

A comparison of the data in figures 7 and 8 shows that on an average the number of microbes in the untreated Palouse soil was about five times greater than that in the untreated Melbourne soil, in spite of the fact that the organic content of the latter as shown in table 1 was nearly twice that of the former, and the total amount of CO_2 produced in the former, as is indicated in table 2, was less than twice that of the latter. When wheat straw or sweet clover hay was added, the microbial population in the Palouse soil was about three to four times that of the Melbourne soil, whereas the total amounts of CO_2 evolved were not much larger in the former than in the latter. Evidently neither the rate of CO_2 evolution nor the soil organic matter content served as a reliable index of microbial numbers.

The data on nitrate nitrogen accumulation, on total CO_2 evolution, and on soil reaction reported in table 2 show that while numbers of microbes in the variously treated samples of the Palouse soil were from three to five times as large as those in identically treated samples of the Melbourne soil, the intensity of activity per unit of microbial population seemed to be greater in the Melbourne soil. Under identical conditions of moisture and temperature the untreated Melbourne soil, with a microbial population about one-fifth as large as that of the untreated Palouse soil, produced more than half as much nitrate nitrogen and CO_2 as the latter. The addition of 1 per cent organic residues with an ample supply of available nitrogen to meet the maximum requirements of the soil microflora during the early and most active stages of decomposition of the residues did not change appreciably the course of these relationships.

In considering the possible cause of these different biological behaviors, the difference in types of microflora in the two soils is noteworthy. As mentioned previously, the dominant groups of microbes in the Palouse soil consisted of bacteria and actinomyces which became still more dominant when organic residues were added. The dominant group in the Melbourne soil, although not shown to be superior in numbers in the untreated sample, appeared to be the fungi, which became more dominant and also superior in numbers when organic residues were added to this soil. Since plate counts for fungi are more representative of the spore content than of the mycelial content, which is the biologically active part in organic matter decomposition, no suitable method is available to compare the work performed by a unit number of bacteria as a group with that performed by a like unit number of fungi. What applies to the fungi with respect to mycelia is also applicable

to the actinomyces, which proved to be about as numerous in the variously treated samples of Palouse soil as were the fungi in comparable samples of the Melbourne soil. Thus, it appears that the cause of the comparatively greater biological activity, including nitrification, observed in the Melbourne soil in relation to microbial numbers is a more energetic activity and greater efficiency in the utilization of released energy by the fungi as a group than by the bacteria or actinomyces as specific groups. The character of the humus in the Melbourne soil is such that ammonification by the microflora composed predominantly of fungi was relatively slow if the fact that the humus content of this soil is nearly twice as large as that of the Palouse soil is considered. When straw or sweet clover hay was added in amounts equivalent to about 12 per cent of the original humus content of this soil, the same microflora was able to ammonify these materials approximately as rapidly as the more numerous microflora of the Palouse soil in which the bacteria and actinomyces predominated. The difference in character of the humus in the two soils

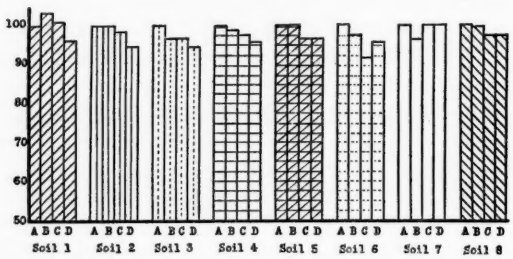

FIG. 10. NITROGEN CONTENT AT DIFFERENT INTERVALS IN PERCENTAGE OF NITROGEN AT THE BEGINNING OF THE EXPERIMENT

A, beginning; B, after 29 days; C, after 35 days; D, after 171 days

rather than the difference in type of the microflora appears to be largely responsible for the observed difference in nitrification in the untreated samples.

The pH values which are recorded in table 2 show that the reaction of the soils became gradually more acid during the course of the experiment. The change in reaction coincided roughly with the progressive accumulation of nitrate nitrogen, which probably was the chief cause of the increasing acidity. In work with soils under field conditions, Stephenson (9) also found changes in soil reaction which coincided closely with nitrate accumulations.

Determinations of the total soil nitrogen content of samples taken periodically showed a small but consistent loss of nitrogen. This is indicated in figure 10, in which the nitrogen content of the various soils is represented in percentage of the nitrogen present at the beginning of the experiment. No significant *Azotobacter* activity occurred in any of the soil samples, and, therefore, no increase in nitrogen content was expected, but the observed loss is difficult to explain on the basis of the available data. A flask containing a standard solution of sulfuric acid and attached at the end of the CO_2 absorp-

tion train never contained more than traces of ammonia, showing that the soils did not release any appreciable quantities of ammonia which could account for the loss of nitrogen.

The data obtained in this study disclose the fact that the native microflora of the Palouse and Melbourne soils which have developed under different climatic conditions and vegetative covers varies distinctly in type. Although the activity of various specific groups of microbes primarily concerned with the transformation of organic matter in these two soils was definitely influenced by the nature and composition of different plant residues, it appeared to be affected to a still greater extent by the inherent soil characteristics including reaction. The intensity of microbial activity as expressed by the production of CO_2 and NO_3, the end products of microbial action, differed markedly in the two soils and appeared to be influenced to a greater extent by the nature of the humus than by the quantity of humus present in the soil. Since soil humus is the product of microbial activity, and this in turn is influenced by the nature and composition of the plant residues that are returned to the soil, it seems that the nature of the vegetation produced under similar temperature and moisture conditions and returned to similar soils or soil materials would result in the production of different types of humus and eventually of soils with different inherent characteristics. This would be applicable to the development of soil properties in cultivated as well as in virgin soils. Further studies of the behavior of specific groups of soil microbes under different conditions in soils with divergent properties are in progress and will be reported in forthcoming papers in this series.

SUMMARY

A study was made of the rate of decomposition of different kinds of plant residues and their influence on the microbial activity in Palouse silt loam and Melbourne silt loam. The plant residues were supplemented with sufficient ammonium nitrate to make the total supply of nitrogen equivalent to 3.18 per cent of their dry weight. The rate of decomposition was ascertained by periodical measurement of carbon dioxide evolution and nitrate nitrogen accumulation; and the microbial activity, by periodical plate counts for bacteria, actinomyces, fungi, aerobic cellulose-decomposing bacteria, and *Azotobacter*.

A distinct difference was found in the native microflora of the two soils. The total number of microbes was about five times as large in the Palouse soil which contained 4.4 per cent of organic matter as it was in the Melbourne soil which had an organic matter content of 8.1 per cent. The microflora of the Palouse soil consisted of a relatively large number of bacteria and actinomyces and a very small number of fungi, whereas that of the Melbourne soil was composed of approximately seven times as many fungi and only about one-sixth as many bacteria and actinomyces as that of the Palouse soil.

The addition of 1 per cent of wheat straw and sweet clover hay respectively

caused a preponderant increase in microbial activity in both soils, as did the addition of like amounts of pine needles and coniferous forest duff to the Palouse soil. All groups except the cellulose-decomposing bacteria and the *Azotobacter* were affected. The numbers in the latter two groups were very small, and, therefore, their activity was not significant.

Although the activity of the same specific groups of organisms in the two soils was not affected alike by the same plant residue, and the influence of different residues on the activity of the various groups of microbes in both soils varied, the relative order of predominance of specific groups of microbes in each of the two soils remained similar. This predominance appeared to be controlled to a greater extent by specific inherent soil characteristics than by the nature of the organic food supplied by the plant residues.

No distinct sequence in activity of specific groups of microbes was manifested in the decomposition of various plant residues, but the fact that the stimulated growth of the fungi persisted for a longer time than that of the bacteria and actinomyces indicated a tendency in this direction.

Increased rates of CO_2 evolution did not coincide with increased microbial numbers as indicated by plate counts, for the maximum CO_2 production resulting from the addition of plant residues preceded the maximum numbers of microbes by a period of about 15 days. A second peak in microbial numbers developed at a time when the rate of CO_2 evolution was very low.

When the fact is considered that the humus content of the Melbourne soil is about twice as great as that of the Palouse soil, the relatively small amounts of CO_2 and NO_3 produced in the untreated sample of the former appeared to be attributable to the character of the humus rather than to the type of microflora. The rate of CO_2 evolution and NO_3 accumulation in identically treated samples of the two soils was not proportional to the microbial population of the samples. The less numerous microflora in the Melbourne soil was capable of decomposing the added organic residues about as rapidly as was the more numerous microflora in the Palouse soil.

The reaction of the variously treated soil samples became gradually more acid as time progressed. The change in reaction coincided roughly with progressive accumulations of NO_3 in the soil. A small decrease in total nitrogen, which could not be accounted for by escaping ammonia through the CO_2 absorption train, took place in all the samples.

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BOOK REVIEWS

Weather Elements. By THOMAS A. BLAIR. Prentice-Hall, Inc., New York, 1937. Pp. xv + 401, illus. 107. Price $4.

The growing interest in climatology is a response to the better understanding of the economic significance of weather and climate. Air navigation has become an important economic phenomenon. The use of the recreational facilities offered by our parks, forests, lakes, and seacoasts calls for a better interpretation of weather forecasts. Food production and distribution must reckon, to an ever-increasing extent, with weather and climate—particularly in view of the widening competition in the national and international trade. For this and other important reasons, the author's contribution to the subject is most welcome.

In writing the book, the author was guided by a definite purpose, as indicated in the following paragraph taken from the preface:

> This book aims to present, concisely and systematically, an introduction to the science of meteorology in its present stage of development. My primary purpose is to set forth the facts and principles concerning the behavior and responses of the atmosphere in such a way as to enable the reader to acquire an elementary understanding of the physical processes underlying observed weather phenomena. An important secondary object is to present that general body of information about the weather and the present state of our knowledge concerning it, which it is believed that every intelligent person should possess in relation to this most important element of his environment.

The book is made up of 14 chapters, 4 appendixes, and an index. The illustrations add in a substantial way to the value of the book. They will be appreciated by the reader and the student. The respective chapters and indexes are designated as follows: The Atmosphere; Observing Temperature, Pressure and Wind; Observing Moisture, Sunshine, Visibility, and Upper Air Conditions; Solar Radiation and Its Effects; Condensation of Water in the Atmosphere; Inter-relations of Temperature, Pressure and Wind; The General Circulation; The Secondary Circulation; Lesser Disturbances; Weather Forecasting; World Weather; Climate; Climate and Man; Electrical and Optical Phenomena; The United States Weather Bureau; Bibliography; Conversion Factors and Tables; Mean Monthly and Annual Temperatures and Precipitation (Tables), and Climatological Section Centers of the United States Weather Bureau.

The presentation is clear and logical and will serve as a basis for the further development of an important field of scientific exploration.

Handbook of Chemistry, ed. 2. By NORBERT ADOLPH LANGE. Handbook Publishers, Inc., Sandusky, Ohio, 1937. Pp. xvi + 1780. Price $6.

This is a revised and enlarged edition of a reference book which has met with wide favor. The first edition was published in September, 1934. The author notes in his preface: "This book is the result of a number of years' experience in the compiling and editing of data useful to chemists." The author also notes, in the preface to the first edition:

> While the principal value of the book is for the professional chemist or student of chemistry, it should also be of value to many people not especially educated as chemists. Workers in the natural sciences—physicists, mineralogists, biologists, pharmacists, engineers, patent attorneys and librarians, are often called upon to solve problems dealing with the properties of chemical products or materials of construction. For such needs this compilation supplies helpful information and will serve not only as an economical substitute for the costly accumulation of a large library of monographs on specialized subjects, but also as a means of conserving the time required to search for information so widely scattered throughout the literature.

The second edition contains much new material. Because of the new material—particularly tables—the size of the volume has been increased by 237 pages. The additions and revisions have come as a result largely of suggestions that have been offered by the users of the *Handbook*. The table of contents is made up of a list of the very many topics discussed in the book and a mathematical appendix. This appendix consists of two parts: Part I is devoted to Formulas and Theorems from Elementary Mathematics, Frontispiece, Greek Alphabet and Mathematical symbols and Abbreviations; Part II is entitled "Tables."

Altogether, the present volume is a rich source of indispensable information. The author and his associates have done their work well and have made a fine contribution in an important field of theoretical and applied chemistry.

Annual Review of Biochemistry, vol. VI. Edited by JAMES MURRAY LUCK. Annual Review of Biochemistry, Ltd., Stanford University P. O., Calif., 1937. Pp. ix + 708. Price $5.

This is the sixth volume of the series. It obviously maintains the high standards set by its predecessors. The magnitude of the editor's task is well indicated in the preface. The editor and his associates—Doctors C. L. Alsberg, D. R. Hoagland, and C. L. A. Schmidt—have unquestionably earned the graditude of many workers in the field of biochemistry.

It may be worth while to quote the following from the preface:

> The preparation of a preface to each succeeding volume of the Review is somewhat more than a routine task of which we may dutifully but, nevertheless, lightly dispose. We choose to regard it as an occasion to report upon changes in editorial policy, but even more as a pleasurable opportunity whereby we may join with our readers in paying tribute to the writers of these reviews. Their task has called for the exercise of judgment, discrimina-

tion, and forbearance under circumstances of unusual difficulty. Year by year severe restrictions have had to be imposed upon the lengths of the various reviews. Many substantial works have had to be placed aside—a circumstance which is trying to an author, if not vexatious. The tide of papers of biochemical interest continues to rise but only a fraction can be selected for review. Over 10,000 abstracts of papers in biological chemistry appeared in *Chemical Abstracts* in 1936. The science has lost none of its youthful vigor. Even a casual reader cannot fail to be impressed by its lively activity, by the enthusiasm with which new regions are explored, and by the wealth of discovery which follows upon fundamental investigations in scattered fields.

The subjects dealt with in the *Review* for 1937, and the names of the contributors, follow: Permeability—R. Collander; Biological Oxidations and Reductions—F. Lipmann; Enzymes—K. Linderstrøm-Lang; The Application of Microchemistry to Biochemical Analysis—P. L. Kirk; The Chemistry of the Carbohydrates and the Glycosides—W. N. Haworth and E. L. Hirst; The Chemistry of the Lipins—E. Klenk and K. Schuwirth; The Chemistry of the Steroids—R. Schoenheimer and E. A. Evans, Jr.; The Chemistry of the Proteins and the Amino Acids—G. S. Adair; The Chemistry and Metabolism of the Compounds of Sulfur—V. du Vigneaud and H. M. Dyer; Chemistry and Metabolism of the Nucleic Acids, Purines, and Pyrimidines—F. Chrometzka; Carbohydrate Metabolism—H. J. Deuel, Jr.; Fat Metabolism—R. G. Sinclair; The Metabolism of Proteins and Amino Acids—S. Edlbacher; Detoxication Mechanisms—A. J. Quick; The Hormones—G. F. Marrian and G. C. Butler; The Vitamins—C. C. Sherman and H. C. Sherman; Nutrition (Energy Metabolism)—M. Kleiber; The Biochemistry of Muscle—D. M. Needham; The Metabolism of Brain and Nerve—R. W. Gerard; The Biochemistry of Fish—C. M. McCay; Chemical Embryology—D. M. Whitaker; Plant Pigments—J. H. C. Smith; The Alkaloids—E. Späth; Photosynthesis—R. Emerson; Mineral Nutrition of Plants—F. G. Gregory; Organic Acids of Plants—T. A. Bennet-Clark; Biochemistry of Bacteria—C. B. van Niel; Immunochemistry—K. Landsteiner and M. W. Chase. There are also an author index and a subject index. These indexes are not only well done but they serve to enhance our amazement at the almost startling growth of the literature in one of the newer fields of scientific exploration.

Many workers in the field of chemistry will find both pleasure and satisfaction in adding the sixth volume to an already impressive-looking shelf.

Soil Conditions and Plant Growth, ed. 7. By E. JOHN RUSSELL. Longmans, Green and Co., London—New York—Toronto, 1937. Pp. viii + 655, plates 11, figs. 65. Price $7.

The seventh edition of this important work should be in great demand among members of the staffs of our experiment stations and agricultural colleges. The author's well-known facility for interpreting and popularizing facts and generalizations in the field of soil and plant science helps to make this book as readable as its predecessors.

The author refers to the fact that the new developments in soil science have made necessary a far-reaching revision of the book. He notes:

> Its purpose, however, remains unaltered; it is to present the student with the broad outlines of the subject, including sufficient detail to give reality to the treatment but avoiding always the tediousness of the card-index record. The numerous references have been so selected that they at once lead the student into the literature of the particular problem. Additional space was of course needed for the new material, but I have been able to reduce in other directions so as to avoid increasing the size of the book: I do not wish it to grow too large; it is intended to be read, not merely consulted like a dictionary.

The book contains 8 chapters, an author index, and a subject index. The topics dealt with under the several headings are as follows: Historical and Introductory; Soil Conditions Affecting Plant Growth; The Composition of the Soil; The Soil in Nature: I. Changes in its Mineral Composition; The Soil in Nature: II. The Changes in the Organic Matter; The Micro-organic Population of the Soil and its Relation to the Growth of Plants; The Biotic Conditions in the Soil; and Soil Fertility in Nature and in Farm Practice.

Soil Conditions and Plant Growth, as formerly, will serve effectively both as a text and as a reference book. It will continue to be a favorite among teachers in American colleges and universities and workers in our experiment stations. The author's large audience will feel grateful to him for having brought up-to-date much important information in a rapidly expanding field of human knowledge.

ABC of Agrobiology. By O. W. Willcox. W. W. Norton & Company, Inc., New York, 1937. Pp. 323, figs. 21. Price $2.75.

The author has written in the same field on the subjects of "Reshaping Agriculture," "Nations can Live at Home," and "Can Industry Govern Itself?" In the preface to the present volume, the author tells us: "If the most important subject that can engage the interest of mankind is the study of man himself, it is surely of next importance to understand the natural means on which man must rely for his very existence."

The guiding thought of the author in preparing this book is partly indicated in the following paragraph quoted from the preface:

> The fundamental yardsticks of plant growth and yield, without which there can be no quantative science of plant life, have been supplied by the modern agrobiologist. "Quantity of plant life" can now be gauged on a definite scale and "Baule units of growth factors" have become established concepts which anybody can understand and use if he is interested in plants. The upshot is that plant culture, when placed under an agrobiologic regime, becomes an exact—even a stringently mathematical science, by which the end may be foreseen from the beginning; and it is now known that the quantitative end-results of plant culture may be vastly greater than the old plant biology ever suspected.

The chapters of the book have the following titles: What is Agrobiology?; The Indivisible Kingdom of Plants; Thinking It Out; The First Agrobiologists at Work; Setting up the Scale of Soil Fertility; The Fertility Index; Using

the Scale of Soil Fertility; Sources of Frustration; The Agrobiological Evaluation of Water; The Stand of Plants; The Quantity of Plant Life; More About the Formula 318/N; Looking at the Limits; Public Soil Science in the United States; Review of the Basal Axioms; Agrobiologic Equilibria or Endstates; and Mathematical Details. There is an appendix entitled "Hints on Fertilizers" and an index.

The author's views and interpretations will provoke much thought, even though the student of plant nutrition may not always agree with the author in the far-reaching conclusions which he has drawn. Certainly, it must be admitted that the returns from our land are far below those which can be obtained within the limits of economic production. In order to supply the raw materials for human and animal nutrition and the raw materials for our industries, we, obviously use more land than is really necessary. The implications of this are so significant that the thought-provoking exposition of the author should be accepted with due appreciation.

Silicate analysis. By A. W. GROVES. Thomas Murby & Co., London, 1937. Pp. xxi + 230, illus. 11, tables 8. Price 12/ 6 net.

The student of soil science will feel more than ordinary appreciation to the author for the contribution he has made by this book. The very general interest in soil conservation and in the phenomena of erosion and leaching should make the present volume all the more welcome. Studies are now under way in many lands on the genesis and development of soil types, the changes in the soil profile, inventory and balance-sheet of plant nutrients. There is a rapidly growing interest in the so-called *minor* constituents of the mineral portion of the soil. More exact appraisal of the composition of the soil is one of the pressing needs of soil and plant science. The author is well aware of the significance of accuracy in making silicate analyses. He tells us in the preface:

> The analysis of rocks and silicate minerals is a matter of prime importance not only to the geologist and mineralogist, for such analyses are required with growing frequency for technical and commercial purposes. While analyses carried out with a technical or commercial aim are usually not so detailed as those for scientific research, the need for accuracy is generally no less. Whatever the end in view, the tendency is to stipulate more constituents than formerly. The purpose of this book, therefore, is to assist both chemists and geologists in the attainment of a high standard of silicate analysis.

There are included within the limits of the book a preface, foreword by Prof. Arthur Holmes, and likewise a table of contents and a list of illustrations. The 12 chapters are designated, respectively, as: The Laboratory: Its Equipment and Apparatus; Reagents; Sampling and Crushing; Constituents to be Determined in Rock Analysis: Limits of Error; Common Operations; Normal Methods for Silicate Rocks; Special Methods; Notes on Technological Applications and other Special Cases; Errors; Qualitative Tests to Decide Whether Certain Constituents are Worthy of Determination; Occurrence of the Various

Elements; and Computations as a Check on the Accuracy of Chemical Analysis. There are three appendixes, designated as follows: (a) Factors; (b) Specimen Calculation of Analysis; and (c) Statement of Analysis.

The arrangement of the subject matter, the explicit directions, and the clear presentation will make this volume all the more useful to the worker in the chemical laboratory.

Foundations of Silviculture upon an Ecological Basis, ed. 2. By JAMES W. TOUMEY, revised by Clarence F. Korstian. John Wiley & Sons, Inc., New York, 1937. Pp. xxi + 456, illus. 22. Price $4.50.

The first edition of this book was published in 1928. Since the death of Professor Toumey, the work has been revised by Clarence F. Korstian of Duke University. The extent of the revision is indicated in the preface to the second edition. The author says:

> In the revision of "Foundations of Silviculture," which was undertaken at the suggestion of Professor Toumey just prior to his death in 1932, a conscious effort has been made to retain as much of his original material as recent advances in forestry, ecology, and plant physiology justify. These advances have made it necessary to reorganize and rewrite some of the chapters and to add new material. No attempt has been made to cover all of the recent advances, but rather to select material illustrative of the particular principles to be emphasized.

The book is divided into three parts, designated, respectively, as Environment of the Forest; Influence of the Forest on its Environment; and The Forest. There are, aside from the preface to the second edition and the preface to the first edition, 18 chapters, the titles of which follow: Introduction—Definitions and Generalities; Solar Radiation; Air Temperature; Atmospheric Moisture; Climate; Soil Conditions; Soil Moisture and Its influence on Forest Vegetation; Physiographic Conditions; Biotic Factors; Interaction of Site Factors; Reaction of Forest Vegetation on its Physical Environment; Effect of Forests on Animal Life, Particularly Mankind; The Tree; Differentiation and Development of Stands; Reproduction, Growth, and Yield of Stands; Tolerance; Forest Vegetational Units and Their Classification; and Origin and Development of Forest Communities: Forest Succession. These are followed by an appendix dealing with common and technical names of trees, a bibliography, and an index.

The value of this standard work on silviculture has been enhanced by the revision. Our forestry schools should attract more students. The activities of the federal, state, and municipal governments are helping to stimulate interest in our national forests, state forests, and parks. The broad meaning of forests and of silviculture as factors in our national economy, as well as in sociology, are more keenly appreciated than ever before. Hence, the present volume should be a welcome addition to our not too extensive list of treatises on silviculture and ecology.

Soil Science Society of America, vol. I. Edwards Brothers, Inc., Ann Arbor, Michigan, 1937.

The organization of the International Society of Soil Science at the Rome meeting in 1927 has stimulated interest in soil science research in Europe and North America. There has been a growing interest in the subject also in the Far East and in some of the South American countries. Many of the soil workers in the United States were members of the American Society of Agronomy. The increase in the number of workers and progress in specialization gradually brought about the recognition that the American Society of Agronomy should organize in a more definite way its programs relating to crops and soils respectively. After years of discussion, the soil workers in the United States finally organized the Soil Science Society of America. The present volume consists of the papers presented at the first meeting of the society. That meeting was held in Washington, D. C., in November, 1936.

The material presented has been brought together by a committee consisting of Doctors Emil Truog, C. E. Millar, F. J. Alway, and Richard Bradfield, Chairman. It may be worth while to quote the statement of the editors as it appears in the preface:

> At their annual meeting in Washington, D. C., November 17–20, 1936, the Soils Section of the American Society of Agronomy and the American Soil Survey Association decided to merge and to form the Soil Science Society of America. A desire to eliminate the serious overlapping and duplication of the activities of the older organizations was responsible for the change. The main objective of the new organization is to foster all phases of Soil Science. To this end Sections, corresponding in the scope of their activities to the Commissions of the International Society of Soil Science, have been organized. Papers dealing with all phases of Soil Science will be presented at the annual meetings, and will be published in a volume of Proceedings. While the union of the two older organizations was not officially consumated until the Washington Meeting the officers coöperated so closely in their planning that a unified program resulted. The present volume contains the papers presented under the joint auspices of both organizations, together with the constitution and plans for the future of the new organization. The new series of Proceedings, of which this is the first volume, will supersede the Bulletin of the American Soil Survey Association. It will appear annually as soon as possible after the annual meeting. Each volume will contain a complete record of all papers presented and of all business transacted at that annual meeting. It is hoped that this new publication will present each year, in one volume, a fairly complete picture of current American thought on all phases of Soil Science.

The report contains a statement entitled: "Soil Science as Related to Other Sciences." This is followed by groups of papers arranged in six sections, as follows: Soil Physics; Soil Chemistry; Soil Microbiology; Soil Fertility; Soil Genesis, Morphology and Cartography; and Soil Technology. In a joint meeting of Sections 5 and 6, there was a discussion of soil science and land use. The report makes reference also to business meetings.

It should be noted here that the Soil Science Society of America is affiliated with the International Society of Soil Science. Its members recognize the universality of the physical, chemical, and microbiological processes occurring

in soils. The vast literature on the subject that is being built up by workers in many lands is brought nearer home through the efforts of the organized soil scientists. Because of this organized effort, the translation of scientific data into farm practice is greatly hastened. The present volume will, no doubt, be followed by others in the course of years that will tell of the unfolding of soil science in the United States and elsewhere.

Soils of the Lusitano-Iberian Peninsula. By EMILIO H. DEL VILLAR, translated by G. W. Robinson. Thomas Murby & Co., London. Pp. 416, illus. 28, tables 87, with a colored map. Price 40/.

Through the efforts of Dr. G. W. Robinson, Professor of Agricultural Chemistry at the University College of North Wales, this important treatise on the soils of Spain and Portugal has been made accessible to the reader who has no command of the Spanish language. As is noted by Dr. Robinson:

> The material presented by Prof. del Villar in "Soils of the Lusitano-Iberian Peninsula" has an importance which reaches beyond the boundaries of the region with which it deals. Since the realm of pedology is universal, the pedologist who is unacquainted with the soils of other countries has only a partial view of the subject and cannot understand the wider relationships of the material which he studies in his own country.

Both the author and the translator have made a significant contribution in the field of soil science, and have thereby rendered more than an ordinary service to soil workers in many lands. The chapters, the titles of which are given both in English and Spanish, are designated in the former language as follows: Classification and Nomenclature; Region of Acid-Humic Soils; Siallitic Soils; Calcareous Soils; Areas of Calcareous Soil With Decalcified Enclaves; Siallitic-Calcareous Mosaic and Calcified Soils; Saline Soils; Hydropedic Soils; and Epilogue.

Studies like those reported in this book serve to amplify our knowledge of soil types and soil resources of the world. Within the present decade, new exploration has given us soil maps of the Far East, of Eastern and Western Europe, of North and South Africa, of Australia, and of the two Americas. The present work will help to broaden the horizon of the soil scientist and of the agronomist.

JACOB G. LIPMAN.

THE COMPOSITION AND STRUCTURE OF SOIL ORGANO-MINERAL GELS[1] AND SOIL FERTILITY

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THEORETICAL CONSIDERATIONS

The history of the evolution of the theory of soil colloids reveals that soil colloids were once elaborately characterized by their qualitative and quantitative content of exchangeable bases. Wiegner, Gedroiz, and others based their work on this characterization. The theories of Gedroiz were successfully applied to the genetic classification of soils, but when they were applied to the determination of the difference in the fertility of two adjacent plots a positive result was not always obtained. Table 1, which is from Kudzin (11) and which gives the results of the determination of the absorption capacity of adjacent chernozen plots of different degrees of fertility, shows that the total content of exchangeable cations does not reflect the fertility of the different plots; that is, the yields from the plots are distributed in an order different from that of the absorption capacities of the plots.

Besides the Gedroiz method, the molecular ratio SiO_2/R_2O_3 of soil colloids was suggested as a measure of fertility. As Reifenberg (17) has shown, the SiO_2/R_2O_3 ratio is adequate for a general genetic classification of the soils of the United States, but it can not serve as a criterion to differentiate the fertility of two adjacent plots. Van der Marel (42) has demonstrated that the soils of Holland and of Dutch India, showing equal molecular SiO_2/R_2O_3 ratios in their colloids, bind phosphates differently.

It is probable that the characterization of soil colloids by Mattson (14), that is, according to the ratio of acidoids to basoids, may prove to be the most useful. It is to be regretted that the method has not been tested in agricultural practice.

In recent years, with the advancement of our knowledge of the organic fraction of the soil, attention has been directed to this soil constituent. Some of the methods advanced by Springer (28, 29, 30), Waksman and Stevens (43), Simon (24, 25, 26), and others in the study of organic matter may be

[1] The term "gel" is used here as a synonym of "coagel." Neither of these terms, however, is entirely suitable. As can be seen from the text, this paper deals with original colloidal organo-mineral precipitates, for which there is, as yet, no suitable short designation.

[2] The spelling of the author's name, hitherto given in SOIL SCIENCE as "Tiulin," has been corrected to the form used here.

utilized in the study of the importance of organic colloids in the soil, even though the studies of these investigators were not concerned with the colloid reactions of the organic matter.

The method of Simon, involving the isolation of organic matter from the soil by the use of sodium fluoride, is of particular interest. Gedroiz (7, p. 143), isolated from the soil the so-called humate part of the soil-absorbing complex by the use of sodium chloride long before Simon did. However, the salting out of a certain part of the colloids containing mineral substances, as practised by Simon, must be regarded as an advantage. This salting out process, introduced by Oden (16, p. 65), who used, among other salts, sodium sulfate, enabled Simon to obtain relatively pure solutions of humic acid. This constitutes a great advance over the method proposed by Gedroiz. Neither Gedroiz nor Simon, however, considers the forms of combination of the organic colloids with the mineral substance of soil. Special attention is given this question by Springer (30), but, as will be seen, we cannot entirely agree with this author's interpretation of the form of combination of organic matter in the

TABLE 1

Total exchangeable cations on chernozem plots at the Mironovo Agricultural Experiment Station

FERTILIZER	ABSORPTION CAPACITY	YIELD OF SUGAR BEETS
	m.e.	*cwt./ha.*
Without fertilizer	26.8	128
Manure	26.3	214
Mineral fertilizer plus manure	24.7	279

soil. The valuable service of Simon and Springer in the elaboration of the methods of evaluating organic matter in the soil has not as yet been fully appreciated.

The method of Waksman concerns the raw fraction of humus only, that is, cellulose, hemicellulose, and lignin. Raw humus undoubtedly plays an important part in soil fertility, and in this respect, the method of Waksman may be valuable as a supplement to those methods by which the humified fraction or organic matter is determined.

The aforementioned investigations indicate that success in applying the theories of soil colloids to agricultural practice may be anticipated from determinations on the various forms of organic matter and of organic colloids in the soil. The actual colloids in the soil are not, however, a mixture of organic and mineral colloids, but the product of deep-seated reactions between them, as pointed out by Barbier (1). The final results of this interaction are specific organo-mineral gels, found in the soil only. The term "organo-mineral gel," being generally vague, indicates only the peculiar chemical composition of soil colloids and conveys no idea of the structure of these specific double-colloid systems. We know, however, that the chemistry and physics of

colloids are primarily the chemistry and physics of their surfaces (both exterior and interior). We know also to what extent the properties of a gel depend on its structure and, particularly, on the composition and structure of its surface. The literature contains much valuable material on the structure of clayey minerals (3, 9, 12), some on the structure of humic acid (20), and very little on the structure of the organo-mineral gels of soil.

Work on the structure of the organo-mineral gels has been systematically conducted in the Soil Colloid Laboratory of the All-Union Fertilizer Research Institute in Moscow since 1932. The initial aim of this work was to point out the lack of homogeneity of the gels in the soil, even in an individual soil sample; in other words, it was deemed necessary first to dispel the erroneous notion that colloids in the soil are homogeneous. Van Bemmelen (41), however, had already noted that the colloids of clay were nonhomogeneous and could be distributed in two groups. Later, Sokolovskii (27) expressed the same view in regard to soil colloids, which he divided into two groups—active and passive.

TABLE 2

Colloid fractions of podzol profile

HORIZON	SiO_2	Fe_2O_3	Al_2O_3	$\frac{SiO_2}{R_2O_3}$	N	C	LOSS ON IGNITION	ORGANIC MATTER	C/N	NON-ORGANIC MATTER	CATION EXCHANGE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		*per cent*	*m.e.*
A_1	74.00	2.23	14.02	8.15	0.720	18.9	37.00	32.60	27.8	2.2	85.4
A_2	79.00	2.06	14.09	8.75	0.259	4.1	44.60	7.10	15.8	3.5	33.6
B_1	45.40	11.69	35.81	1.79	0.456	7.1	23.90	12.24	15.6	3.7	65.4
B_2	43.20	10.84	21.86	2.55	0.362	7.1	23.40	12.24	19.6	3.0	39.0
C	45.60	10.15	21.67	2.76	0.164	1.36	14.60	2.34	8.3	7.0	21.8

It is scarcely necessary to argue here that the soil mass is always heterogeneous. This motley character of the soil mass, particularly of the arable horizon, is the result of a number of factors. For example, a podzol profile before it has been plowed up, certainly is not homogeneous. Table 2, from *Pedology* by Joffe (8, p. 290), may serve as a good illustration of this thesis. This table shows how sharply the separate horizons differ in all respects. After tillage, some of these horizons will be mixed, giving a still greater complexity of soil colloids. The complexity of the entire soil mass and, in particular, of the soil colloids is accentuated by macroerosion and microerosion, by unequal distribution of fertilizers, and by unequal distribution of the roots, vertically and horizontally.

At this point the question may be raised: In what way can we take an average sample from the arable layer of soil? It may be said that the average sample is "made up" in the laboratory, where the soil is mixed in such a way that it becomes homogeneous. What is actually achieved, however, is an even distribution of a complex material, not an elimination of complexity. In other words, both the colloids in the soil and their surfaces are nonhomogeneous.

Special methods are needed for showing that different groups of colloids do actually exist in the soil. After examining the various methods which might prove useful for this purpose, we chose the behavior of the different gels in any one soil sample toward the same peptizing agent.

If one uses the sodium ion of a sodium chloride solution as the peptizing agent (the Gedroiz method), he should first isolate from the soil those gels that yield to such an ionic or electric peptization. The experiment shows, indeed, that different amounts of colloids may be isolated from different soils by the Gedroiz method; a large quantity is thus isolated from chernozem and a small quantity from red soil, notwithstanding the fact that red soil contains more colloids than does chernozem. After the isolation of colloids by this method, the presence of the residual colloids in the remainder of the soil can be detected readily by the methods of colloidal chemistry and by direct optical methods. An effective indicator of the difference between those colloids that can be isolated by means of ionic peptization and those that cannot be thus isolated is found in the electrokinetic potential. Whereas the electrokinetic potential of the isolated colloids saturated with the sodium ion is about 40 or 50 millivolts, that of the residual colloids, also saturated with the sodium ion, is only about 3 or 4 millivolts, as has been demonstrated by Davydov.[3] Those colloids that can be isolated by the Gedroiz method are called, therefore, "electronegative gels," or gels of the first group, and those colloids that are not isolated by means of ionic peptization are called "isoelectric gels," or gels of the second group.

In order to isolate the so-called "isoelectric gels" from the soil, dissolutive peptization (2, p. 251) was brought about in two stages. The soil remaining after the isolation of the first group of gels was first treated with a solution of 0.004 *N* NaOH, which leads to the isolation of a certain quantity of colloids of the second group. This isolation is not complete, however, because soils contain very aged gels, which are isolated by the following method, which constitutes the second stage of peptization. The remaining soil is treated with weak hydrochloric acid until the mobile sesquioxides are eliminated and then with 0.01 *N* alkali, after which it is washed with distilled water, then with weak alkali and again with water followed by weak alkali. Isolation of colloids by groups is stopped at this point, since the use of stronger peptizers might result in the transformation of some noncolloidal soil components into colloids.

The results obtained by this technic of fractional peptization are described in the following section.

EXPERIMENTAL RESULTS

The two groups of gels were isolated from three distinct types of soils, and the results are presented graphically in figure 1. It can be seen from this

[3] Unpublished report.

graph that the gels of the first group, the electronegative gels, predominate in chernozem and those of the second group, the isoelectric gels, predominate in red soil and in podzol.

Even a quantitative consideration of the gel groups throws some light on the individual properties of certain soils. Thus, the absorption capacity of red soil is very low (about 20 m.e.), although its total content of colloids reaches almost 80 per cent. The reason for this is evident from figure 1. The low absorption capacity of red soil is caused by its low content of gels of the first group, which is mainly involved in the cation exchange.

In our studies, the individual gel groups were investigated qualitatively as well as quantitatively. First, the absorption capacity of the cations was

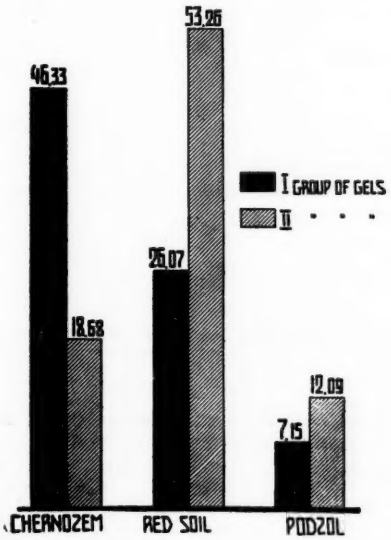


FIG. 1. RELATIVE PROPORTIONS OF TWO GROUPS OF COLLOIDS IN DIFFERENT SOILS

determined both in the original soils and in each of the gel groups separately. Table 3 summarizes these determinations. As can be seen from this table, the absorption capacity of the first group of gels is considerably higher than that of the second group, although the second group was isolated from the soil by means of weak alkali.

From American researches it might be thought that the difference in the absorption capacities of the first and second groups of gels depends on the molecular ratio SiO_2/R_2O_3 in soil colloids. The results of the determination of the molecular ratio SiO_2/R_2O_3 of soil colloids in chernozem and podzol (table 4) show, however, that this is not a satisfactory explanation in regard to chernozem and that it is only partly satisfactory in regard to podzol. As

has already been demonstrated by Mattson (13), however, the molecular ratio SiO_2/R_2O_3 is not an entirely suitable criterion of the absorption capacity. It would be more nearly correct to take into account not only silicic acid but also other acidoids and, primarily, the organic acids and phosphoric acid.

Carbon, nitrogen, and phosphorus were determined in the original soils and in each gel group separately. The results are shown in table 5. It is evident from this table that the amount of organic matter in the second group

TABLE 3

Exchange capacity of chernozem and podzol soils and of their gel groups, on a dry-matter basis

MATERIAL	EXCHANGE CAPACITY
	m.e.
Voronezh chernozem (original)	65.0
First group of gels	90.10
Second group of gels:	
Subgroup *a*	30.24
Subgroup *b*	28.44
Residue after isolation of two groups	16.41
Podzol "Ochakovo" (original)	9.93
First group of gels	36.60
Second group of gels:	
Subgroup *a*	20.66
Subgroup *b*	15.30
Residue after isolation of two groups	1.97

TABLE 4

Silica-sesquioxide ratio of soil colloids in chernozem and podzol soils

MATERIAL	SiO_2	R_2O_3	PERCENTAGE RATIO SiO_2/R_2O_3
Chernozem (original)	63.20	14.47	...
First group of gels	43.62	26.05	3.6
Second group of gels, subgroup *a*	39.81	24.95	3.5
Podzol (original)	69.32	10.04	...
Frist group of gels	52.30	37.92	2.8
Second group of gels, subgroup *b*	30.23	32.33	2.0

is greater than that in the first group; in other words, the total amount of silicic acid and of other acidoids cannot be used as a criterion of the adsorptive properties of colloids.

Leaving aside provisionally the question of the method by which the localization of organic matter in the organo-mineral gels was determined, we shall cite here data demonstrating the mobility of silicic acid and of sesquioxides in the separate gel groups. This mobility was determined by the use of Tamm's reagent (a mixture of oxalic acid with ammonium oxalate at pH 3.2).

The results are given in table 6. As this table shows, the sesquioxides and the silicic acid in the colloids isolated from chernozem are very soluble in Tamm's reagent, whereas those in the original chernozem are only slightly soluble; those in the first group of gels isolated from podzol are as slightly soluble as are those in the original podzol, whereas those in the second group are much more soluble than those in the original podzol.

TABLE 5

Carbon, nitrogen, and phosphorus content of chernozem and podzol soils and of their gel groups

MATERIAL	C	N	P_2O_5
	per cent	*per cent*	*per cent*
Voronezh chernozem (original)	4.80	0.255	0.218
First group of gels	7.00	0.440	0.404
Second group of gels:			
Subgroup *a*	8.64	0.010	0.017
Subgroup *b*	14.88	0.372	0.274
Podzol "Ochakovo" (original)	0.98	0.092	0.113
First group of gels	0.373	0.392	0.588
Second group of gels:			
Subgroup *a*	4.49	0.245	0.035
Subgroup *b*	6.74	0.989	1.22

TABLE 6

Solubility, in Tamm's reagent, of silicic acid and sesquioxides in chernozem and podzol soils and in their gel groups

Percentage on a dry-matter basis

MATERIAL	SiO_2	R_2O_3
	per cent	*per cent*
Voronezh chernozem (original)	0.67	1.42
First group of gels	3.54	9.43
Second group of gels, subgroup *a*	6.09	9.60
Podzol "Ochakovo" (original)	0.38	1.52
First group of gels	1.12	1.66
Second group of gels, subgroup *a*	3.68	4.79

The results summarized in table 6 do not explain the different absorption capacities of the separate gel groups; nevertheless, they indicate some inherent relationship between the amount of organic matter in the colloids and the solubility, in Tamm's reagent, of sesquioxides and of silicic acid. This relationship, insofar as chernozem is concerned, can probably be explained as follows: Table 5 shows that the first and second groups of gels contain large amounts of organic matter. This organic matter, in our opinion, protects the sesquioxides from dissolution in Tamm's reagent when the gels remain intact in the soil. When, however, colloids are isolated from the soil by means

of peptization, both ionic and dissolutive, the protective organic matter on the surfaces of the colloids is disturbed to some extent, and as a result, the dissolution of sesquioxides becomes possible. The first group of gels isolated from podzol contain only an insignificant quantity of organic matter (table 5); no appreciable increase, therefore, occurs in the solubility of sesquioxides in Tamm's reagent. On the other hand, the second group of gels isolated from podzol contain a considerable quantity of humus, and here a marked increase in the solubility, in Tamm's reagent, of sesquioxides and silicic acid occurs.

The foregoing observations strengthened our conviction that the key to the understanding of many of the properties of soil colloids is to be sought in the structure of the organo-mineral gels and, particularly, in the localization of organic matter in the entire mass of gels.

Schloesing (19) many years ago indicated the existence of organic films on the surface of soil colloids. Later work by Fickendey (5), Oden (16, p. 65), and others showed how this protective film of organic matter is formed on the surface of mineral particles.

The method of fractional coagulation, first applied by Schloesing, was used to isolate that part of the organic matter which is not strongly attached to the entire mass of organo-mineral gels. The method, as applied to chernozem, was as follows. Suspensions of the first group of gels, isolated by the Gedroiz method of ionic peptization, were alkalized to pH 8.5 in order to liberate that part of the organic matter least strongly held by the colloids. To the solution thus obtained, 10–15 gm. of KCl per liter was added. This caused an immediate precipitation. The supernatant liquid, which was of a cherry-red color, contained the organic matter least strongly held by the colloids. This fraction is apparently Simon's so-called "humic acid." The KCl treatment of the liquid was repeated until the humic acid was completely isolated. The latter was then coagulated by the use of HCl, and the carbon, nitrogen, and phosphorus content and the cation absorption capacity were determined. From the precipitate formed as a result of the KCl treatment, additional organic matter was isolated, as follows. The precipitate was first treated with weak HCl, to destroy the bond between the organic acids and the sesquioxides, and then with weak alkali, which produced a dark-colored solution. By the addition of KCl, as above, to this solution, the second fraction of organic matter was obtained. The precipitate remaining after the isolation of the first and second fractions of organic matter still contained a considerable quantity of organic matter. Attempts to isolate this by means of hydrolysis with stronger alkali and by means of prolonged treatment with pyridine were unsuccessful; in other words, this organic matter fraction was strongly attached to the mineral part of the colloids.

The results obtained by the determinations just described are shown in table 7. As can be seen from this table, the absorption capacity of the first fraction of humates (or "humic acid," according to Simon) is exceptionally high, 416.6 m.e. per 100 gm; and that of the second fraction of humates is also

high, 338.4 m.e. The absorption capacity of the first group of gels as a whole was only 81.08 m.e., and after the elimination of these two humate fractions, the absorption capacity of the residue decreased to 47.7 m.e. These data show clearly, therefore, that the loosely combined fraction of humates represents the outer border layer in the first group of colloids.

Emphasis should be placed on the fact that the properties and behavior of the first group of gels from chernozem are determined to a considerable extent by a small amount of organic colloids situated at the surface of the gels. A second conclusion, which also should be emphasized, is that the loosely combined humates have a high nitrogen content and a fairly high phosphorus content, both of which are probably important from the standpoint of soil fertility.

TABLE 7

Chemical characteristics of the first group of gels and of original chernozem

MATERIAL ANALYZED	TOTAL QUANTITY	ABSORPTION CAPACITY	C	N	P_2O_5
	per cent	*m.e.*	*per cent*	*per cent*	*per cent*
First group of gels	100	81.08	5.31	0.74	0.295
First fraction of humates	2.72	416.6	46.23	4.67	0.352
Second fraction of humates	3.78	338.4	41.4	3.24	0.723
Residue	93.50	47.7	2.46	0.158	0.101
Original soil		45.4	3.25	0.294	0.143

TABLE 8

Chemical characteristics of the second group of gels isolated from chernozem

MATERIAL	ABSORPTION CAPACITY	C	N	P_2O_5
	m.e.	*per cent*	*per cent*	*per cent*
Subgroup *a*:				
First fraction of humates	178.3	34.63	....	0.64
Second fraction of humates	180.0	38.07	....	2.20
Residue	37.1	1.91	....	0.14
Subgroup *b*	250.0	48.43	3.34	1.48

The characteristics of the second group of gels isolated from the same chernozem are shown in table 8. The absorption capacities of the humate fractions of subgroup *a* are considerably lower than those of the corresponding fractions of the first group of gels, but the phosphorus content is higher. In this connection, it must be pointed out that some of the raw humus of the soil may have been partly hydrolyzed. The absorption capacity of subgroup *b* is somewhat lower than the absorption capacities of the two humate fractions of the first group of gels, but the phosphorus and nitrogen contents are high.

On the basis of colloidal chemical determinations alone it would be difficult to say how the organic substance of the second group of gels is held with reference to the mineral substance in these gels. One can only assume that the organic matter is not loosely held by the mineral part but is probably the result of a more intimate reaction with the mineral fraction of the gels.

The foregoing results led to the following conclusions concerning the structure of organo-mineral gels in chernozem:

Gels of the first group have an organic matter fraction within them where it is very firmly held by the mineral fraction. Another fraction of organic matter is found on the surface of the gels and is held less firmly, or even loosely.

In the second group of gels, either organic matter does not occur at the surface of the gels or, because of some influences, such as dehydration and chemical bonds, it is unable to manifest the exchange capacity of cations as in the first group of gels.

The second group of gels may be more active in the adsorption of anions than is the first group. This question has been considered elsewhere (33).

OPTICAL METHODS IN THE STUDY OF COLLOIDS

The methods of colloidal chemistry are inadequate to reveal the exact structure of the gels; consequently, research workers began some time ago to use optical methods in the study of colloids. Thus, Fry (6) determined microscopically the quantity of colloids in soil, and Kubiena (10) studied under the microscope the shape of soil formations. Of special interest in connection with the structure of soil organo-mineral gels are the recent optical studies of soil structures reported by Sideri (21, 22, 23), who discovered crystallike formations which are of a type hitherto unknown in soils and which resemble the paracrystalline formations of Rinne (18). In the light of the data and conclusions of Sideri, it is evident that the so-called "organo-mineral gels" are not common gels or coagels.

Thus, new possibilities of research are opened by the optical methods. By combining, in future, the methods of colloidal chemistry and the optical methods, research workers will probably obtain very valuable information on the structure of soil organo-mineral gels. At present, however, the quantity of organic matter loosely held by the organo-mineral colloids is the only new criterion of soil fertility that can be used.

PRACTICAL APPLICATIONS

The quantity and the composition of the organic matter loosely held by the first group of gels, at least those of chernozem, must be taken into account before we can hope to solve successfully the practical problems referred to in the beginning of this article. The first attempt at such a practical application was made in our laboratory by a collaborator, A. F. Skvortzov,[4] who investi-

[4] Work not yet published.

gated by our method two plots of chernozem at the Suma Agricultural Experimental Station, each of which exhibited different degrees of fertility. One of these plots was manured and the other was not. As was to be expected, the yields on the manured plot were higher than those on the unmanured plot. Skvortzov isolated the first group of gels from samples taken from each of the plots and determined the quantity of the first fraction of humates. He found that this fraction from the manured plot equaled 20.05 gm. per kilogram of soil and that from the unmanured plot equaled only 16.6 gm., a difference of about 25 per cent. In the laboratory of the Sugar Beet Research Institute, G. K. Davydov, applying the same method to other chernozems, obtained similar positive results.

From these results, we conclude that the proposed method for evaluating soil fertility will be practicable after it has been considerably simplified. An attempt at simplification will be undertaken next.

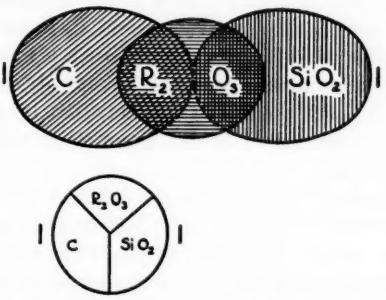


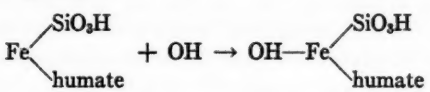
FIG. 2. COMPOSITION AND STRUCTURE OF SOIL COLLOIDS

The figure below is another form of representing the figure above. The vertical lines outside the figures represent the minus charges of the particles.

DISCUSSION

The practical application of the theory of soil colloids to agriculture is limited by our lack of knowledge of the structure of soil gels, of the forms of combination of organic matter with mineral substance in these gels, and of the composition of this organic matter. Indeed, in earlier works the structure of soil colloids is described in only the most general way.

A few years ago, the author represented the composition and structure of soil colloids in the manner shown in figure 2 (31). Later, Mattson (15) proposed the following scheme for the structure of soil colloids, greatly resembling the one shown in figure 2:



Springer (27), as already noted, considered in more detail the forms of combination of organic matter in soil. Perhaps the most interesting ideas on this problem, however, were advanced by Schloesing (19). His ideas were correctly evaluated by Ehrenberg (4, p. 123–131), who attempted to draw the attention of research workers to them.

Our own researches (31, 33–40) support the theory of Schloesing as to that part of the organic matter which is loosely attached to the entire mass of organo-mineral gels. Besides loosely attached humus, however, these gels contain considerable quantities of organic substance that is more firmly held. The nitrogen and phosphorus of the humus that is strongly attached to the entire mass of gels are probably almost unavailable to microorganisms; therefore, the loosely held humus is of greater importance from the standpoint of soil fertility.

Many properties of soil depend on the organic matter content of its gels. This fact enhances the significance, as a factor of fertility, of the organic substance of organo-mineral gels. It is essential, therefore, that a simpler technic be devised for the differentiation of these gels into groups and for the isolation of loosely held humates from each group. It seems easy to suggest a simplified technic, both direct and indirect, that is, without isolation of colloids, for the determinations of the quantitative relations between the various gel groups in soil.

It should be expedient to utilize for the isolation of loosely held humates all the methods elaborated by Simon and Springer. It is true that Springer (29) criticized our method, doubting the possibility of isolating passive gels from soil. The basis for this observation was found in an earlier article (32), which treated only superficially the question of loosely held humates and the structure of gels. The present article, in which the nature of soil gels and the forms of combination of organic matter in these gels are considered in greater detail, disposes of Springer's criticism.

There are possible other objections, however, to the method used in separating the colloids from the soil. The literature contains many indications of denaturation of colloids in the course of their isolation and of transition of coarsely dispersed systems into colloids during this process. All these observations are, however, of a very general nature. The various authors make no attempt to analyze the principles of the different methods of peptization. Some of these methods, however, have recently been considered scientifically, for example, in Von Buzágh's book *Kolloidik* (2, p. 251). On the basis of modern views on peptization and of the available data on the structure of soil gels, it may be said that the Gedroiz method of ionic peptization does lead to a denaturation of colloids. We can now say more precisely to what kind of denaturation. Where amorphous humus is present at the surface of the gels, for example, in gels isolated from chernozem, ionic peptization may break off some of this humus and transform it to a free state. The result of such

denaturation can be predicted, and it can be calculated quantitatively and qualitatively following the reaction. The second type of peptization, the dissolutive type, naturally causes a profounder denaturation of soil colloids. Since our purpose, however, is to determine soil fertility by isolating from separate groups of gels the humate that is loosely attached to these gels, the denaturation of these colloids in the process is not of great importance. The amount of humate and the nitrogen and phosphorus content of the humate can be determined here with considerable accuracy. The danger is that treatment even with weak alkali may lead to the hydrolysis of some raw humus. In this respect, the results may be corrected by the use of acetyl bromide, which dissolves raw humus but does not dissolve humified organic matter.

Our work has clarified our understanding of how peptization takes place and of what happens to the colloids in this process. We understand better why such large amounts of colloids are isolated from chernozem after treatment with sodium chloride. The cause lies in the special rôle played by the film of amorphous humus on the surface of the particles. This amorphous humus is readily hydrated after saturation with the sodium cation, becomes highly charged, and when separated from the particle carries with it everything lying below it.

What has been said concerns chernozem; each of the other soil types possesses peculiarities, which cannot, however, be discussed here.

A principle of classification for soil colloids can be formulated from the results of our researches; this is of great theoretical and practical importance.

SUMMARY

Soil colloid research has produced noteworthy results during recent years. The practical utilization of these results, however, is still very limited. The author believes that this is due to lack of knowledge of the special properties of soil colloids, such as the properties of organo-mineral gels, which have a peculiar composition and structure, and particularly to disregard not only of the organic fraction but also of the nitrogen and phosphorus content of that part of the organic matter that is most loosely attached to the soil colloids.

The work described in this paper establishes, by the use of the methods of fractional peptization and of fractional coagulation, the nature of the organo-mineral gels in soil. It demonstrates the presence of loosely held humus at the surface of such gels and the significance of this humus for the evaluation of soil fertility.

The quantity and the composition of the loosely held humates in soil colloids may serve as a valuable criterion of soil fertility or of the extent to which the soil can be cultivated.

For practical use, a simpler technic than that now available is needed for determining the humates which are loosely held by the soil gels. This is the next goal of research in this field.

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TITRATION CURVES AND DISSOCIATION CONSTANTS OF SOIL ACIDOIDS

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Titration curves furnish very important sets of values for expressing the characteristics of acids. Bradfield was the first to point out a close analogy between soil acidoids and true acids based on the general similarity of titration curves of weak acids (2). He used clay suspensions which were gradually added to alkali. It was argued that points of inflection in the titration curves of weak acids are apparent only when the acid is added to alkali and not vice versa. The clay suspensions used were not electrodialyzed or acid treated, and consequently a portion of the acidoid in them was already neutralized, and the titration curves were incomplete. This defect was removed in later studies in which electrodialyzed clays were used (3). Recently Anderson and Byers have given the neutralization curves of the colloids of soils representative of the great soil groups (1). All these studies refer to the colloidal fraction separated from soils. Very little has been done on soils as a whole.

Titration curves would undoubtedly prove a valuable aid in characterizing soil colloids. The lack of attention so far paid to this method is largely due to the difficulty of obtaining reproducible results. It is well known that the equilibrium between soil and alkali or acid added to it is not established quickly; therefore, the results obtained by the incremental additions of alkali and by pH determination after each addition cannot be relied upon. Increasing amounts of alkali must be added to weighed portions of the soil and the pH determined when equilibrium is established. This fact was recognized by Anderson and Byers (1), who used 36 to 48 hours' shaking to bring about equilibrium.

Natural soils with no preliminary treatment are obviously not suitable for such a study; unless the soil is electrodialyzed or acid treated to remove all the exchangeable base we cannot obtain the complete titration curve. Removal of the exchangeable base is easily accomplished by leaching the soil with 0.05 *N* HCl until the leachate is free from Ca, followed by leaching with water until the leachate is free from Cl ions. All results reported in this paper, unless otherwise stated, refer to acid-treated soils in which the exchange complex is in the acidoid state and no exchangeable base is present.

EXPERIMENTAL

A number of preliminary observations were made regarding the most suitable conditions under which titrations could be done. For this purpose a black

cotton soil of high clay content was used. This soil, on account of its high base-exchange power, was found to be very suitable for standardizing the technic.

Time required for equilibrium between soil and alkali

Increasing amounts of NaOH were added to 5-gm. portions of soil, the total volume of solution being 50 cc. in every case. The pH value of the mixture was determined by the glass electrode, almost immediately and after varying intervals of time. The results plotted in figure 1 show that equilibrium is reached in 48 hours with occasional shaking. To insure the attainment of equilibrium, the soil and base should be shaken for at least 48 hours. In all our studies the soils were shaken in a mechanical shaker for 48 hours with different amount of alkali.

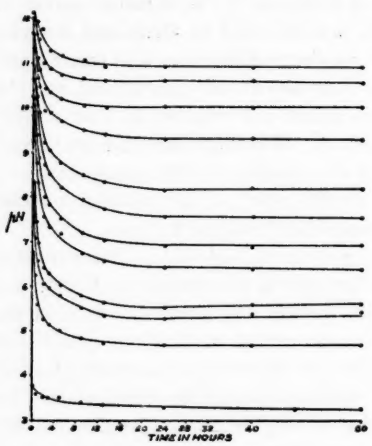

FIG. 1

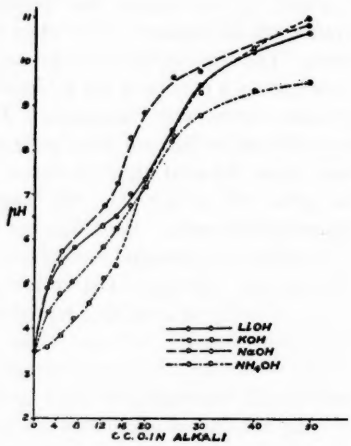

FIG. 2

FIG. 1. SPEED OF REACTION BETWEEN SOIL P.C. 13 A.T. AND SODIUM HYDROXIDE
FIG. 2. TITRATION OF SOIL P.C. 13 A.T. WITH HYDROXIDES OF MONOVALENT IONS

Titration of soil with different bases

The same soil was shaken for 48 hours with increasing amounts of different bases, other conditions being kept the same. The results given in figures 2, 3, and 5 show that the characteristic shape of the titration curves is maintained with every base, although different amounts of alkali are required to produce a particular pH value with various ions. This is in agreement with the observations made on weak acids like phosphoric acid. The actual course of the titration curve depends on the nature of the alkali used, although the end point must be reached with the same amount of alkali in every case. The problem is rather complicated for soils, as the end point is not sharp. The end point must be reached at different pH values in the case of different al-

kalies. In this connection it is important to decide at what pH value the soil should be considered as completely neutralized. The end point of soil, in common with that of the weak acids trails off and therefore cannot be defined. We can, however, make use of a method developed by Harris (5) for the potentiometric estimation of acids and bases which are too weak to be estimated volumetrically. The theoretical principles involved have been discussed by Britton (4, p. 124) and need not be repeated here. The main conclusion on which the method is based is that for weak acids neutralization with a strong base takes place between two definite pH values. In other words, the pH interval between the beginning and the end of a titration for monobasic acids is 4. It is therefore necessary to titrate only between these

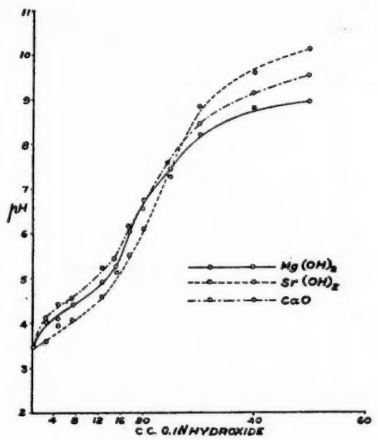

FIG. 3

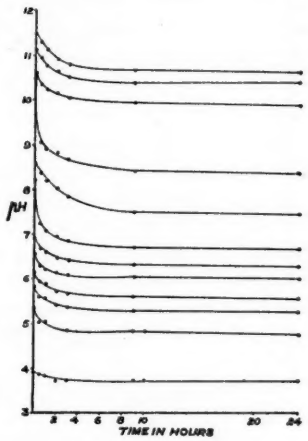

FIG. 4

FIG. 3. TITRATION OF SOIL P.C. 13 A.T. WITH HYDROXIDES OF DIVALENT IONS
FIG. 4. SPEED OF REACTION BETWEEN CLAY ACIDOID AND SODIUM HYDROXIDE

limits to obtain the neutral point. Figures 2, 3, and 5 show that the neutralization point occurs at pH 7.5. It is also seen that although the inflection in the curve occurs at this point the titration is by no means complete. This is a clear indication that we are dealing with dibasic acid. It must be emphasized that the inflection in the curve is even more marked than that in the titration curves of several dibasic acids, such as succinic acid and tartaric acid, and is at least as marked as that in the titration curve of malonic acid. Nothing in these curves shows that acidoids behave any differently from other weak acids. The slowness with which equilibrium is reached is only to be expected from the heterogeneity of the system. Bradfield (2) overcame this difficulty by using a completely dispersed clay suspension which was gradually added to excess of alkali, but there is no doubt that equilibrium is established for soils if we wait long enough.

A closer study of the titration curves of soils would convince anybody that what is known as "base-exchange capacity" of soils has no theoretical significance even if it refers to an arbitrarily fixed uniform pH value. The neutralization point of different acids lies at different pH values, as is true also for soils. *The acidoid equivalent must constitute one of the fundamental constants for characterizing soils.* It could be determined by merely converting the soil into the acidoid and then titrating potentiometrically with NaOH to a pH value that is 4 units higher than the pH of the acidoid. Since it is a dibasic acid this alkali titer must be multiplied by 2 to obtain the acid equivalent. The complete neutralization of the acidoid takes place at such a high pH value

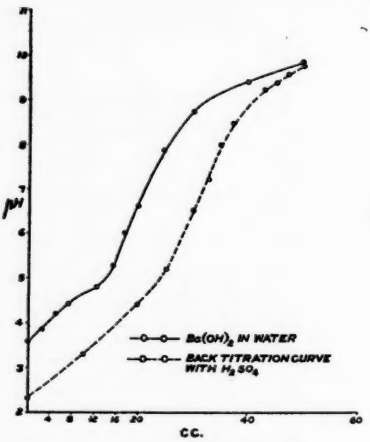

FIG. 5

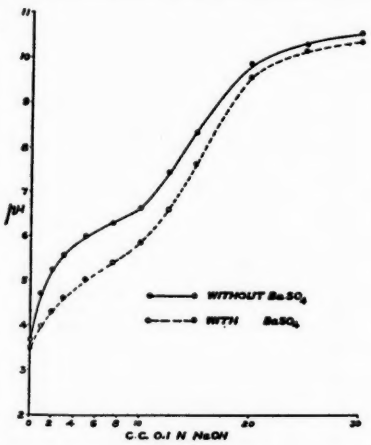

FIG. 6

FIG. 5. TITRATION CURVE OF SOIL P.C. 13 A.T. WITH BARIUM HYDROXIDE

FIG. 6. TITRATION CURVE OF CLAY ACIDOID WITH SODIUM HYDROXIDE WITH AND WITHOUT BARIUM SULFATE

that for all practical purposes only the neutralization of the first H need be considered.

"Exchangeable bases," "exchangeable hydrogen," "base-exchange capacity," and "saturation capacity," unfortunately, are terms that are extensively used in soil literature, and any attempt to discard them completely is likely to be abortive. We can, however, impart to this terminology a more fundamental background by giving the terms a new orientation in the light of the titration curves of soil colloids. Accordingly, the various terms may be interpreted as follows:

Total exchangeable bases in a soil are equivalent to the amount of the acidoid already neutralized with bases, or the saloid in it (S).

Base-exchange capacity is equivalent to the total base required for the formation of mono-acid saloid ($T/2$).

Saturation capacity is equivalent to the total base required for the formation of normal saloid (T).

Exchangeable hydrogen is equivalent to exchange capacity minus total exchangeable bases ($T/2$-S).

The aforementioned interpretations are fundamental and bear no reference to any particular method of estimation. A clarification of the issue, it is hoped, will lead to a greater refinement of the technic in the determination of these constants for the soil. This will result in a better agreement between the various methods, for it must be admitted that when two or more methods for the measurement of a certain constant lead to different results, the error is not in the methods, but in our conception of the constant that we are measuring. Any advancement in the methods of measurement, therefore, can only result from a clear knowledge of what we want to measure. The main difficulty in making soil measurements has been that we measure something first and then give it a name: the measurement changes, but the name sticks.

As shown in the foregoing, the equilibrium between soils and alkalies is attained rather slowly. This is because the soil particles exist in aggregates, and when alkali is added, the bulk of it goes to neutralize the outer surfaces of the particles, with which it first comes in contact. The remaining portion of the soil then slowly takes up alkali from the overneutralized portion until equilibrium is established. That the speed of reaction is dependent on the stage of aggregation is shown by the fact that a completely dispersed clay suspension takes about 9 hours to come to equilibrium, as against 48 hours taken by the soil. This is shown in figure 4.

Back titration curve of single-base soils

The back titration curve of a Ba-soil with H_2SO_4 is given in figure 5. It will be seen that the back titration curve is lower then the forward curve but that in other respects it is similar. The fall in pH value at every stage is due to the slight solubility of $BaSO_4$, which lowers the pH value by suppressing the ionization of the saloid (3). This explanation is supported by the fact that the titration curve of a clay suspension with NaOH showed a similar shift when pure $BaSO_4$ (Pro Rontgen) was added to it. This curve is included in figure 6. It is a remarkable fact that even the slight solubility of $BaSO_4$ should cause such an appreciable shift in the pH value. The results might seem to throw some doubt on the colorimetric method of Kuhn (6) for determining pH values in the presence of excess $BaSO_4$. In actual practice, however, probably no such shift in the pH occurs, because ordinary soils already contain sufficient quantities of salts to have caused the initial lowering of the pH value, and the added amount of $BaSO_4$ would not cause much further change. It is only in the absence of salts that even the slightest addition would produce change.

Titration curves of soils

Titration curves of 65 soils were determined. Approximately 15 gm. of soil were acid treated, leached with water and then with alcohol, and air dried. One-gram portions of the treated soil were shaken for 48 hours with 10 cc. of NaOH solutions of increasing concentration. The pH values were measured with the glass electrode. Thus titration curves of six to eight soils were determined in one lot. *Each one of these curves showed an inflection at point approximately 4 pH units higher than that of the original acidoid.* Some of these curves that could be accommodated together are given in figures 7 and 8. The acidoid equivalents of these soils ($T/2$) were determined by interpolation at $(a + 4)$ when a is the pH value of the acid-treated soil. These

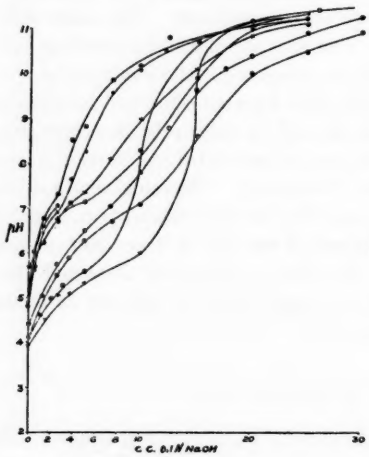

FIG. 7

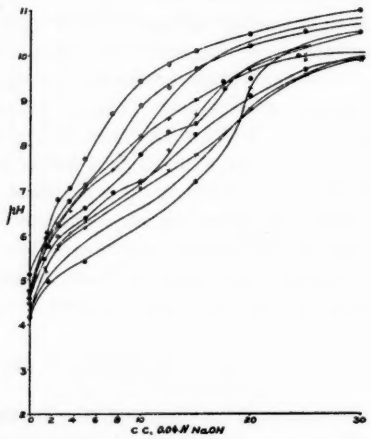

FIG. 8

FIG. 7. TITRATION CURVES OF VARIOUS SOIL ACIDOIDS

FIG. 8. TITRATION CURVES OF VARIOUS SOIL ACIDOIDS

values, which may be termed the "base-exchange capacities," are given in table 1, together with the clay content of the soils. The $T/2$ values per 100 gm. of clay are also included in the table and could be used for characterizing clays in different soils, provided we are prepared to believe that the acidoid is present only in the clay fraction. It is extremely unlikely, however, that this is the case. Clay refers only to an arbitrary size and as such cannot be expected to include the whole of the acidoid present in the soil. This question, however, will be examined in another paper. It must be emphasised that $T/2$ and T values must not be confused; the former, termed "base-exchange capacity," is actually measured, the latter, termed "saturation capacity," is retained for theoretical reasons on account of the dibasicity of the

TABLE 1

Acidoid equivalent ($T/2$) of soils and their pK values

SOIL NO.	CLAY	PER 100 GM. SOIL $T/2$	$T/2$ PER 100 GM. CLAY	pK
	per cent	*m.e.*	*m.e.*	
M. 1	32.2	23.20	72.0	6.68
2	13.76	12.00	87.2	7.32
3	22.60	14.60	64.6	7.35
4	8.70	12.80	147.1	7.37
5	11.46	20.00	174.5	7.35
6	16.00	10.00	62.5	7.30
7	13.90	28.00	201.4	8.70
8	29.80	28.20	94.6	6.42
9	9.26	12.40	133.9	7.30
10	25.26	16.60	65.7	6.40
11	19.10	14.00	73.3	7.10
12	18.34	16.20	88.5	6.60
13	8.98	9.40	105.1	7.58
14	15.80	10.60	67.3	6.53
15	25.68	13.50	52.7	6.67
16	5.84	5.80	100.0	7.06
17	14.60	10.00	68.5	7.25
18	33.28	15.60	46.9	6.80
20	20.72	7.90	38.2	7.12
21	8.94	14.20	159.3	5.65
22	2.32	12.30	531.0	7.20
23	16.32	12.40	76.5	6.80
P.C. 1	11.30	12.00	106.2	6.81
P.C. 2	59.30	54.40	91.7	4.90
3	62.20	61.00	98.1	5.00
5	19.30	10.56	54.7	6.70
6	28.40	11.80	41.5	7.18
7	21.80	7.30	33.4	7.82
8	25.20	19.60	77.8	5.78
9	21.60	7.70	35.5	7.30
10	35.60	19.80	55.6	5.63
11	32.80	26.00	79.3	5.49
12	7.20	4.90	67.8	7.25
13	58.90	40.00	67.9	6.40
14	21.50	24.00	116.3	6.75
15	22.40	5.70	25.4	7.55
17	14.10	6.48	46.9	6.50
20	8.10	3.80	46.9	8.28
21	13.50	11.44	84.7	6.65
25	4.00	3.76	94.0	9.75
26	21.50	7.68	35.7	8.43
27	53.20	50.40	94.7	4.87
28	49.60	23.00	51.6	5.07
29	63.00	43.60	69.2	4.75
30	54.10	49.20	90.9	5.17
31	22.80	12.96	56.8	6.43

TABLE 1—*Concluded*

SOIL NO.	CLAY	PER 100 GM. SOIL $T/2$	$T/2$ PER 100 GM. CLAY	pK
	per cent	*m.e.*	*m.e.*	
33	2.60	7.76	298.5	9.25
39	8.40	9.60	114.3	6.55
40	31.10	9.86	31.7	6.15
44	8.40	6.48	77.1	6.83
45	11.10	5.28	47.6	6.50
48	19.80	5.44	27.4	6.75
49	27.30	14.08	15.6	6.15
50	17.70	7.52	42.5	6.70
51	22.23	6.40	28.8	6.57
52	11.31	7.52	66.5	6.65
53	17.60	6.64	37.7	6.80
54	3.20	5.68	177.5	7.10
55	11.00	10.64	96.7	7.31
56	13.13	9.36	71.3	6.45
57	4.45	5.92	133.0	7.00
58	13.43	7.28	54.2	6.78
60	7.37	8.00	108.5	6.35
61	.....	11.52	.....	6.50
123	8.08	15.60	19.2	6.07

Acetic acid	4.7
Uric acid	5.8
Hypochlorous acid	7.4
Boric acid	9.2
Phenol	10.0

acidoid: the former is more important from the practical point of view, the latter is necessary for maintaining a proper perspective of the true state of affairs.

Dissociation constants of soil acidoids

The titration curves of weak acids having dissociation constants of less than 10^{-4} with strong alkalies like NaOH are defined by the usual mass law equation

$$pH = pK + \log \text{salt/acid}.$$

Hence when the acid is half neutralized, i.e., the ratio salt/acid is unity, pH = pK. Thus the pH value of a weak acid when it is half neutralized with NaOH is equal to the logarithm of the reciprocal of its dissociation constant. Just as pH is used for comparing the intensity of acidity of different solutions, pK values could be used for comparing the activity of the acids so that the higher the value of pK the weaker the acid. It might be noted that although the pH values of soils change with the content of exchangeable bases, the pK values are fundamental constants that could be used for characterizing soils.

Such values determined from their titration curves for various soils are given in table 1. The pK values of some common acids are also given for comparison.

It will be seen that some soil acidoids are as strong as acetic acid, the majority are only slightly weaker than uric acid, and only very few are as weak as boric acid.

If the dibasicity of soil acidoids is assumed, then these values should be denoted as pK_I, referring to the dissociation constant of the first hydrogen atom. The dissociation of the second hydrogen atom is so feeble that the normal saloids would be very strongly hydrolyzed. The pK_{II} values, therefore, can be of only theoretical interest and are difficult of determination. It would be advisable, however, to consider the pK values as referring to the dissociation of the first hydrogen atom.

It is also worthy of note that in soils we might be dealing with a mixture of acidoids of slightly differing pK values, but in the absence of specific information on this point it would be convenient to consider that we are dealing with one acidoid having a definite pK value determined by the titration curve.

Titration curves of soils have not been introduced extensively in the routine analyses of soils so far. These measurements as a basis of soil classification should receive greater attention at the hands of soil scientists. By the use of the glass electrode and 0.05 *N* acid treatment, as many as 10 soils could be examined in one lot.

SUMMARY

Titration curves of soil acidoids closely resemble those of weak dibasic acids. The point of inflection occurs approximately 4 pH units above the initial pH of the acidoid and corresponds to the neutralization of the first hydrogen.

Dissociation constants of soil acidoids can be determined from their titration curves. The pK values of soils are fundamental constants that refer to the activity of soil acidoids. The smaller the pK value, the stronger is the acidoid.

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SOME PHYSICAL AND CHEMICAL PROPERTIES AND THE KIND OF ORGANIC MATTER AFFECTING COLOR IN RANDALL CLAY AND UPLAND SOILS OF THE SOUTHERN HIGH PLAINS[1]

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Information concerning the texture, the total nitrogen, and the kind of organic matter in soils of the southern high plains is very limited as compared with the amount of data that has been published on soils in more humid regions. Russel and McRuer (23) found that texture is the outstanding factor in virgin grassland soil determining the nitrogen and organic matter content. Russel (22) also reports that a general relationship of these constituents is commonly accepted in soils under various climatic conditions. Brown and O'Neal (5), Marbut (18), and Shaw (27) generally concluded that the organic matter content is not dominant in determining color of soil and that it occupies a very minor place in determining the characteristics of soil profiles. Rice (21) states that the geographic region in which a soil occurs, the topographic features, the position of the colored horizon in the profile, and other correlated characteristics must be known before the color can be accurately evaluated and that in land classification the color must be interpreted in relation to environment and to the other features of the profile. Other investigators (13, 16, 22, 25, 28, 29) have shown that the factors affecting the accumulation of organic matter and of nitrogen in soils are temperature, precipitation, aeration, oxidation, latitude, length of growing season, and any biochemical or physical process which influences microorganic life.

Organic substances with different carbon-nitrogen ratios were added to waterlogged soils by Sreenivasan and Subrahmanyan (26), who found that during the process of fermentation the carbon and nitrogen were lost from the soil system. According to De and Sarkar (10), when soils that contain a ratio of available carbon-nitrate nitrogen of 30–50:1 are waterlogged, the

[1] Contribution from the Oklahoma Agricultural Experiment Station, Stillwater, Oklahoma. The research was conducted at the Panhandle Agricultural Experiment Station, Goodwell, Oklahoma.

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nitrate will be rapidly assimilated by the microorganisms, but when the proportion of carbon is less, a portion of the nitrate is dentrified.

Much information has been published (6, 15, 20, 35, 36) on the nature of organic matter, but some of the investigators do not agree on the rate of decomposition of the end products. Waksman and his associates (31, 35, 43) have reported that lignins are more resistant to decomposition than are the polysaccharides and proteins and tend to accumulate. Oberholzer (19) measured CO_2 production and concluded that rate of decomposition was in the order of glucose, lignin, cellulose, and starch. Smith and Brown (30) state "that lignin does not possess antiseptic properties and though it may decompose slowly, it gradually disappears from soils." Vandecaveye and Allen (33) found that lignin in wheat straw remained unchanged for a period of 156 days on Palouse silt loam. Under anaerobic conditions (32) lignin in corn stalks, in rye straw, and in alfalfa decomposed very slowly, and that in oak leaves, not at all. It is quite evident that lignin gradually decomposes (36, 37, 43), but since it is resistant to the action of microorganisms (20) and even in the presence of weak alkaline solutions absorbs oxygen, forming a dark-colored pigment, this residue has been described (39, 40, 41, 42) as having all the properties of soil "humus."

The humus in chernozem soil (38) was characterized as being fixed by calcium and magnesium and as having a narrow carbon-nitrogen ratio, a high lignin and protein content, and a low percentage of cellulose and hemicellulose. The carbon-nitrogen ratio in chernozem soil (3) was less than that in soils of the humid area and decreased with depth, but it was very constant in the surface layer, in view of the wide variation in carbon. A close relation was found (1, 14) to exist between the carbon and nitrogen content of soil, the ratio of nitrogen-carbon being about 1 to 10. According to Allison (1), when a heavy application of organic fertilizer was made to a soil, the carbon was consumed by organisms until a constant ratio was established, but if this ratio was narrow in the added organic matter, the organic matter was attacked by ammonifying organisms, and free ammonia was produced.

EXPERIMENTAL PROCEDURE

Composite samples from the first and second foot of soil were collected from areas of Randall clay which have developed on lacustrine deposits, and from typical well-drained upland soils around old lake beds, in the Panhandle of Oklahoma and Texas in 1936. Fitzpatrick and Boatright (12) found that the areas of Randall clay in Texas County, Oklahoma, are circular or oval in shape, range from 10 to 500 acres in size, and have a very dark gray or black surface. This fine-textured black soil is underlain by a white lime zone at a depth ranging from about 2 to 4 feet. They state that "This soil is probably better supplied with organic matter and plant nutrients than any other soil in the county, but it is not very productive because of its heavy texture and because it is often covered with water."

The soil samples were air-dried, pulverized, and analyzed for total nitrogen, total carbon, carbonate carbon, and manganese according to methods recommended by the Association of Official Agricultural Chemists. An aliquot taken from the solution prepared for the determination of manganese was analyzed for iron according to a procedure recommended by Daniel and Harper (8). The organic matter was determined by the chromic acid method as recommended by Schollenberger (24). The percentage of sand, silt, and clay was estimated by the hydrometer method (4), using sodium hydroxide and sodium oxalate as a dispersing agent. The composition of soil organic matter was determined by the proximate simplified method used by Waksman and Stevens (44).

RESULTS

Relation between soil texture, total nitrogen, and organic matter in Randall clay and upland soil

The results of the sand, silt, clay, total nitrogen, and organic matter analyses of all soils are recorded in table 1. The areas of Randall clay contained considerably more clay and less sand than did the well-drained upland soils. With the exceptions of samples 484, 545, 561, and 568, the first foot of soil in the lake bed contained less total nitrogen and organic matter than did the adjacent upland. Since these samples were collected from an area that has been badly eroded by wind and since the surface foot of these soils contains less clay and more sand than does the second foot in the same profile, this condition may be due to the accumulation of drifting soil in these lake beds. The clay areas contained 29.7 per cent less sand, 41.3 per cent more clay, and 51.3 per cent more hygroscopic moisture than did the upland soils when the surface foot in each of these areas was compared. All lake bed soils had a very dark gray or black surface and a very fine-textured black clay deposit varying in depth from about 2 to 4 feet. Regardless of texture and color, these soils contained an average of 27.1 per cent less total nitrogen and 27.7 per cent less organic matter than did the soil around these areas. No outstanding difference occurred in the silt content of these profiles. The second foot of Randall clay had an average of 21.7 per cent less sand, 23.9 per cent more clay, 21.5 per cent less nitrogen, 20.0 per cent less organic matter, and 38.3 per cent more hygroscopic moisture than did that of the upland soils.

The coarse-, medium-, and fine-textured soils (7, 9) recorded in figure 1 were taken from areas of typical soil, occurring in the southern high plains, similar to Richfield, Pratt, Potter, and Pullman. The total nitrogen and organic matter in these soils increased with fineness of texture, with the exception of Randall clay, which contained by far the highest clay content and was lowest in these plant nutrients. The average percentage of total nitrogen and organic matter in it is less than that in the coarse-textured soils. These data show that dark color and fine texture are not associated with a high total nitrogen and organic matter content.

TABLE 1

Sand, silt, clay, total nitrogen, organic matter, and hygroscopic moisture content of Randall clay and of near-by well-drained upland soil in the southern high plains

SAMPLE NO.	LOCATION	FOOT LAYER OF SOIL	UPLAND SOIL						RANDALL CLAY					
			Mechanical analysis			Chemical analysis			Mechanical analysis			Chemical analysis		
			Sand	Silt	Clay	N	O.M.*	H_2O†	Sand	Silt	Clay	N	O.M.*	H_2O†
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
466	4 mi. E. Guymon, Okla.	1	45.0	28.2	26.8	0.086	1.43	1.25	19.8	25.0	55.2	0.084	1.34	2.72
		2	40.4	25.8	33.8	0.054	0.84	2.35	19.0	27.8	53.2	0.057	0.84	3.38
484	4 mi. E. 1½ S. Guymon, Okla.	1	34.2	24.6	41.2	0.085	1.17	1.66	23.8	23.4	52.8	0.092	1.48	2.09
		2	20.2	35.6	44.2	0.055	0.73	3.57	20.8	24.4	54.8	0.058	0.82	4.14
502	1 mi. W. 1½ mi. N. Goodwell, Okla.	1	32.2	27.8	40.0	0.075	1.16	3.30	23.9	26.9	49.2	0.068	1.09	4.17
		2	28.8	31.0	40.2	0.071	1.00	3.34	24.9	27.1	48.0	0.049	0.79	3.28
516	5 mi. S. 2 E. Conlen, Texas	1	46.2	23.2	30.6	0.078	1.19	3.24	35.8	21.8	42.8	0.060	0.88	2.59
		2	22.8	20.0	57.2	0.064	0.88	4.40	31.6	22.8	45.6	0.056	0.69	5.19
524	5 mi. S. 4 W. Hooker, Okla.	1	55.4	11.4	33.2	0.112	1.72	1.71	45.0	18.6	36.4	0.079	1.45	3.72
		2	52.8	17.6	29.6	0.060	0.87	1.62	49.2	17.4	33.4	0.049	0.72	4.44
530	1 mi. E. Stevens, Texas	1	42.6	28.6	28.6	0.134	2.16	2.02	44.8	17.6	37.6	0.058	0.93	2.43
		2	45.2	23.6	31.2	0.076	1.10	3.34	49.2	19.6	31.2	0.035	0.48	2.59
545	8 mi. S. 3 W. Conlen, Texas	1	59.2	18.5	22.2	0.087	1.33	1.39	30.8	24.8	44.4	0.099	1.55	2.67
		2	57.8	17.8	24.4	0.050	0.76	1.61	28.5	22.1	49.4	0.058	0.89	3.44
553	6 mi. S. 1 E. Conlen, Texas	1	54.6	22.0	23.4	0.080	1.33	1.41	33.8	23.8	42.4	0.052	0.84	2.16
		2	56.9	13.1	30.0	0.053	0.78	1.95	33.9	22.3	43.8	0.049	0.74	4.09
561	S. C. S. Project, Dalhart, Texas	1	60.0	17.4	22.6	0.072	1.21	1.27	33.8	23.2	43.0	0.084	1.31	3.75
		2	58.1	13.3	28.6	0.058	0.84	3.65	32.4	21.2	46.0	0.049	0.72	4.14
568	10 mi. S. Conlen, Texas	1	61.9	15.3	22.8	0.078	1.55	1.77	33.6	26.8	39.6	0.118	1.86	4.03
		2	56.6	12.2	31.2	0.067	1.03	2.09	29.6	20.6	49.8	0.059	0.86	4.25
		1	45.6	26.2	28.2	0.138	2.33	2.57	20.8	22.6	56.6	0.073	1.05	2.94

584	3 mi. E. 1 W. Conlen, Texas	1	45.6	26.2	28.2	0.138	2.33	2.57	20.8	22.6	56.6	0.073	1.05	2.94
		2	41.2	22.8	36.0	0.080	1.26	2.60	17.8	22.0	60.2	0.063	0.91	4.66
599	1 mi. E. Boise City, Okla.	1	46.4	18.8	34.8	0.104	1.59	1.55	27.0	20.2	52.8	0.059	0.93	4.36
		2	50.1	15.1	34.8	0.059	0.81	2.62	28.0	21.0	51.0	0.052	0.77	4.89
607	1½ mi. S. W. Keyes, Okla.	1	47.9	16.7	35.4	0.114	1.93	2.02	27.6	22.6	49.8	0.060	0.98	2.73
		2	37.2	19.2	43.6	0.078	1.14	3.63	27.9	17.9	54.2	0.045	0.74	4.85
614	7 mi. S. E. Felt, Okla.	1	65.1	16.3	18.6	0.071	1.17	1.51	40.1	38.5	21.4	0.065	1.02	1.78
		2	56.3	18.5	25.2	0.064	0.93	2.44	38.4	42.4	19.2	0.045	0.59	3.93
621	8 mi. N. Eva, Okla.	1	36.6	32.0	31.4	0.097	1.62	1.45	22.0	27.6	50.4	0.074	1.12	2.27
		2	28.1	36.7	35.2	0.063	0.91	1.61	21.6	25.2	53.2	0.056	0.86	5.03
628	1 mi. W. Farnsworth, Texas	1	28.6	36.2	35.2	0.106	1.88	1.58	22.0	34.2	43.8	0.059	0.93	2.14
		2	23.8	30.8	45.4	0.072	1.05	1.99	21.0	32.2	46.8	0.045	0.69	4.60
636	27 mi. S. Texhoma, Okla.	1	40.2	26.0	33.8	0.092	1.31	1.85	30.0	25.4	44.6	0.052	0.79	1.76
		2	27.2	30.8	42.0	0.064	0.97	4.17	33.2	25.2	41.6	0.049	0.72	3.95
644	5 mi. W. Ideal, Texas	1	30.8	26.8	42.4	0.092	1.31	2.16	29.4	23.4	47.2	0.055	0.86	4.06
		2	33.1	25.1	41.8	0.058	0.84	4.24	27.9	24.7	47.4	0.049	0.81	4.37
652	12 mi. S. W. Spearman, Texas	1	26.9	37.9	35.2	0.121	2.19	1.71	25.6	29.2	45.2	0.069	1.07	2.18
		2	26.2	34.0	39.8	0.076	1.21	3.79	24.3	30.5	45.2	0.052	0.79	4.58
660	6 mi. E. Morse, Texas	1	29.2	32.8	38.0	0.101	1.53	1.85	28.9	28.7	42.4	0.057	1.00	1.99
		2	24.5	37.1	38.4	0.072	0.97	4.02	28.7	25.1	46.2	0.052	0.79	4.20
668	10 mi. W. Ideal, Texas	1	29.8	29.8	40.4	0.095	1.34	1.91	46.4	11.4	42.2	0.064	1.09	2.84
		2	25.4	34.6	40.0	0.072	1.00	3.92	49.3	13.9	36.8	0.052	0.81	3.20
Average of all soils		1	43.7	24.6	31.7	0.096	1.550	1.870	30.7	24.6	44.8	0.070	1.120	2.830
		2	38.7	24.5	36.8	0.065	0.950	3.000	30.3	24.1	45.6	0.051	0.760	4.150

* Organic matter determined by the chromic acid method (24).
† Hygroscopic moisture present in air-dried samples.

Some of the Randall clay areas are covered with water most of the time, but these ponds have accumulated a considerable amount of residue as a result of erosion from adjacent upland. A good example of a lake and the surrounding soil is shown in plate 1. This picture was taken after a hard rain, and approximately 90 acres of land is covered with water. In some places where the lakes have been drained or water has been excluded as a result of moisture conservation practices around the areas, buffalo grass grows well. In other areas where this soil is cultivated, the color is still dark gray or black.

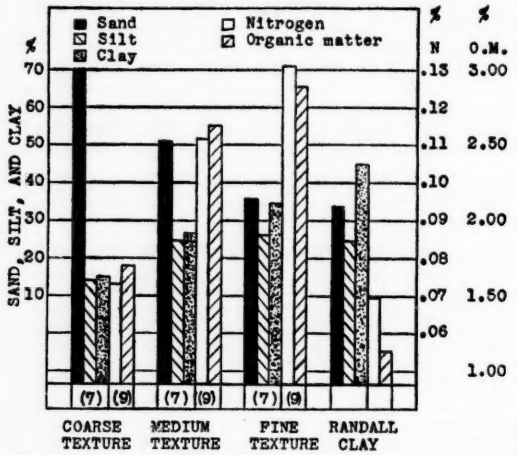

FIG. 1. RELATION BETWEEN TEXTURE AND ORGANIC MATTER AND NITROGEN IN RANDALL CLAY AND IN COARSE-, MEDIUM-, AND FINE-TEXTURED VIRGIN SURFACE SOIL OF THE SOUTHERN HIGH PLAINS

Coarse texture, not more than 20 per cent clay, 24 samples analyzed; medium texture, 20 to 30 per cent clay, 26 samples analyzed; fine texture, more than 30 per cent clay, 11 samples analyzed; Randall clay, 21 samples analyzed.

Nature of organic matter and some inorganic properties of Randall clay and upland soil

In order to study some of the constituents contributing to the dark color in Randall clay, these soils were analyzed for total carbon, carbonates, and organic carbon. The samples showed considerable variation as indicated in table 2, but no outstanding differences existed between the sums of the percentage of carbonate and organic carbon and the total carbon content in these soils. The average percentage of carbon recovered as carbonate and organic carbon from the surface of clay areas was 92.9; and that from the surface of the upland, 95.6. The percentage of carbon not recovered as carbonate or as organic carbon may be considered as inert carbon (2). This difference is probably not significant and may be due merely to the experimental error in methods of determination. The average total carbon content

TABLE 2

Carbon content of Randall clay and of upland soil

SAMPLE NO.	FOOT LAYER OF SOIL	UPLAND SOIL				RANDALL CLAY			
		Per cent carbon				Per cent carbon			
		Total	CO_2	Organic*	Recovered as organic and CO_2	Total	CO_2	Organic*	Recovered as organic and CO_2
466	1	0.86	0.03	0.83	100.0	0.87	0.07	0.78	97.7
	2	0.61	0.11	0.49	98.4	0.70	0.14	0.49	90.0
484	1	0.79	0.01	0.68	87.3	0.99	0.02	0.86	88.9
	2	0.51	0.02	0.42	86.3	0.57	0.01	0.48	86.0
502	1	0.76	0.03	0.67	92.1	0.72	0.06	0.63	95.8
	2	0.70	0.02	0.58	85.7	0.52	0.01	0.46	90.4
516	1	1.07	0.33	0.69	95.3	1.10	0.50	0.51	91.8
	2	4.47	3.89	0.51	98.4	1.12	0.70	0.40	98.2
524	1	2.98	1.86	1.00	96.0	0.87	0.02	0.84	98.8
	2	3.86	3.30	0.50	98.4	0.56	0.06	0.42	85.7
530	1	1.36	0.06	1.25	96.3	0.60	0.01	0.54	91.7
	2	1.65	0.93	0.64	95.2	2.08	1.79	0.28	99.5
545	1	1.34	0.52	0.77	96.3	1.03	0.00	0.90	87.3
	2	0.97	0.52	0.44	99.0	1.17	0.57	0.51	92.3
553	1	0.79	0.01	0.77	98.7	0.53	0.01	0.49	94.3
	2	0.61	0.12	0.45	93.4	0.46	0.02	0.43	97.8
561	1	0.78	0.05	0.70	96.2	1.08	0.27	0.76	95.4
	2	1.67	1.10	0.49	95.2	0.91	0.44	0.42	94.5
568	1	0.99	0.09	0.90	100.0	1.42	0.20	1.08	90.1
	2	0.93	0.29	0.60	95.7	0.92	0.39	0.50	96.7
584	1	1.68	0.26	1.35	95.8	1.25	0.58	0.61	95.2
	2	2.05	1.30	0.73	99.0	1.25	0.70	0.53	98.4
599	1	1.01	0.02	0.92	93.1	0.60	0.00	0.54	90.0
	2	0.53	0.00	0.47	88.7	0.54	0.02	0.45	87.0
607	1	1.61	0.49	1.12	100.0	1.73	1.10	0.57	96.5
	2	2.61	1.90	0.65	97.7	1.69	1.16	0.43	94.1
614	1	0.73	0.02	0.68	95.7	0.61	0.01	0.59	98.4
	2	1.07	0.48	0.54	95.3	0.40	0.00	0.34	85.0

* Determined by the chromic acid method (24).

TABLE 2—*Concluded*

SAMPLE NO.	FOOT LAYER OF SOIL	UPLAND SOIL				RANDALL CLAY			
		Per cent carbon				Per cent carbon			
		Total	CO_3	Organic*	Recovered as organic and CO_3	Total	CO_3	Organic*	Recovered as organic and CO_3
621	1	0.96	0.01	0.94	99.0	0.76	0.01	0.65	86.8
	2	0.81	0.19	0.53	88.9	0.59	0.04	0.50	91.5
628	1	1.19	0.10	1.09	100.0	0.65	0.01	0.54	84.6
	2	0.81	0.12	0.61	90.1	0.47	0.01	0.40	87.2
636	1	0.97	0.11	0.76	89.7	0.54	0.03	0.46	90.7
	2	0.93	0.35	0.56	97.8	0.45	0.01	0.42	95.6
644	1	0.88	0.00	0.76	86.4	0.59	0.00	0.50	84.7
	2	0.59	0.06	0.49	93.2	0.50	0.01	0.47	96.0
652	1	1.31	0.02	1.27	98.5	0.70	0.02	0.62	91.4
	2	0.83	0.04	0.70	89.2	0.52	0.04	0.46	96.2
660	1	0.98	0.01	0.89	91.8	0.59	0.00	0.58	98.3
	2	0.69	0.07	0.56	91.3	0.49	0.01	0.46	95.9
668	1	0.95	0.04	0.78	86.3	0.68	0.00	0.63	92.6
	2	0.71	0.04	0.58	87.3	0.75	0.28	0.47	100.0
Average	1	1.14	0.19	0.90	95.6	0.85	0.14	0.65	92.9
	2	1.31	0.71	0.55	96.2	0.79	0.31	0.44	94.9

of Randall clay was 0.85 per cent as compared with 1.14 per cent in the other soils. The second foot of these soils contained an average of more carbonate and less organic carbon than the surface. With a few exceptions, the lake bed soils contain less total carbon, carbonates, and organic carbon than do the adjacent soils, showing that the dark gray or black color is not due to carbon.

The nature of the organic matter and the iron and manganese content of Randall clay and upland soil were determined. The results are recorded in table 3. Qualitative tests were made on these soils for sulfides, but the quantity was so low that no quantitative data were obtained. Waxes and gums (benzene and alcohol extract), cellulose, protein, and lignin were reported from an analysis of the organic matter content of these soils; the percentage recovered ranged from 81.51 to 99.84. The average percentage of organic matter recovered in the surface soil was 87.72 for the upland soil and 89.93 for the Randall clay. As previously shown (19), the cellulose values may be slightly low because of the presence of iron. Since Waksman and Stevens (44) state that the method used for the aforementioned analysis

gives only approximate values and the percentage of iron was rather constant in these soils, it would seem probable that fairly accurate comparisons may be made between the different soils. There were some variations in the waxes and gums and in the protein content of the individual samples, but all the areas of Randall clay contained more lignin and less cellulose than did the adjacent upland soils. The surface of the lake bed soil averaged 2.35 per cent waxes and gums, 11.64 per cent cellulose, 35.57 per cent protein, and 40.37 per cent lignin; the percentages of these constituents in the upland soil were 2.15, 16.96, 36.82, and 31.79 respectively. The second foot of both types of soil contained more waxes and gums, more protein, more lignin, and less cellulose than did the surface.

Joffe (17), in discussing "regur" soils in India, reported that this black cotton land is deficient in humus and the color is due not so much to the organic matter as to the presence of titaniferous magnetite in the parent material. In order to determine whether total iron or manganese in the Randall clay was responsible for the dark color, analyses for these elements were made. In the majority of samples the percentage of total iron and manganese was higher in Randall clay than in the upland soil. The surface of the former soil contained an average of 2.81 per cent iron and 0.068 per cent manganese, and the percentages in the latter soil were 2.18 and 0.040, respectively. The second foot of these soils contained slightly less iron and manganese than did the surface.

The carbon-nitrogen ratio in these soils is apparently within the normal range (34) of 8 to 12 parts of carbon to 1 part of nitrogen, with an average of about 10 to 1. The lignin and protein (humus) -nitrogen ratios are considerably higher in Randall clay, and the cellulose-nitrogen ratio is greater in the upland soils. The last two ratios in the second foot in both soils are also much higher than those in the surface.

Some of the properties affecting color in Randall clay and upland soil

The color of dry soil is closely connected with the mineral composition of the soil and with varying amounts and kinds of organic matter. Investigators (11, 17) have shown that several factors affect soil color, such as organic matter, iron and manganese compounds, silica, lime, feldspars, and kaolinites. Data given in table 3 show that the dark gray or black clay areas contained more waxes and gums, lignin, iron, and manganese than did the adjacent soils.

Although Randall clay contained more iron and manganese than did the upland soils, the total percentage of these elements was very low. Soluble material was removed by boiling the different soils in a 10 per cent solution of hydrochloric acid; when air-dried soil thus treated was compared with the original sample very little change in color was apparent. Under excess moisture conditions and lack of aeration, protoxides of iron (11) have been found to cause a bluish gray coloring in the soil. Since the organic carbon, determined by digesting Randall clay with a standard chromic acid solution, re

TABLE 3

Nature of the organic matter and the total iron and manganese content of Randall clay and upland soil

SAMPLE NO.	FIRST LAYER OF SOIL	UPLAND SOIL							RANDALL CLAY						
		Per cent organic matter*					Per cent		Per cent organic matter*					Per cent	
		Waxes and gums†	Cellulose‡	Protein	Lignin	Recovered	Fe	Mn	Waxes and gums†	Cellulose‡	Protein	Lignin	Recovered	Fe	Mn
466	1	2.13	17.10	37.23	36.72	93.18	1.86	0.048	2.17	10.68	35.08	42.72	90.65	2.52	0.060
	2	4.25	11.41	38.79	37.25	91.70	2.17	0.036	2.54	6.83	36.49	48.80	94.66	2.65	0.056
484	1	3.00	13.70	39.50	34.40	90.60	2.41	0.052	2.08	12.30	34.30	46.77	95.45	2.92	0.068
	2	3.06	9.88	40.76	35.57	89.27	2.46	0.048	2.97	9.22	37.62	48.90	98.71	2.80	0.088
502	1	1.79	17.84	37.54	40.84	98.01	2.55	0.052	1.95	12.47	34.59	44.21	93.22	2.69	0.068
	2	1.80	13.36	37.90	43.72	96.78	2.72	0.052	2.36	10.91	34.79	51.13	99.19	2.97	0.056
516	1	2.80	14.21	37.65	30.55	85.21	2.27	0.036	3.89	6.39	36.25	42.51	89.04	2.66	0.052
	2	2.66	7.88	39.89	33.58	84.01	1.58	0.028	3.88	5.11	40.59	49.07	98.65	2.99	0.056
524	1	1.79	11.86	36.89	31.88	82.42	1.15	0.012	1.96	7.51	33.18	50.20	92.85	2.42	0.072
	2	2.27	10.29	38.99	33.31	84.86	1.18	0.016	3.54	4.81	40.66	50.56	99.57	2.32	0.070
530	1	1.86	12.96	37.46	36.82	89.10	2.49	0.052	1.40	8.95	35.96	37.96	84.07	2.60	0.064
	2	1.96	12.84	38.48	37.60	90.88	2.05	0.032	1.91	7.40	40.71	43.02	93.04	1.80	0.032
545	1	1.61	16.11	38.47	31.61	87.80	1.40	0.016	2.11	13.50	34.66	40.59	90.86	3.13	0.092
	2	1.86	10.58	40.29	32.37	85.10	1.63	0.028	3.17	3.19	35.20	42.56	84.12	2.98	0.052
553	1	2.18	18.41	37.43	28.94	86.96	2.12	0.028	2.27	13.54	36.20	43.56	95.57	2.82	0.076
	2	2.37	15.67	39.23	29.86	87.13	2.01	0.020	2.28	6.58	40.06	43.58	92.50	2.84	0.068
561	1	2.11	14.95	35.57	30.81	83.44	1.66	0.028	2.01	13.34	37.50	42.59	95.44	3.04	0.048
	2	2.14	11.16	40.45	34.13	87.88	1.46	0.028	2.29	10.90	38.11	48.54	99.84	2.70	0.044
568	1	2.43	19.56	31.35	29.27	82.61	1.54	0.028	3.11	13.98	35.16	35.06	87.31	2.91	0.056
	2	3.98	15.41	37.69	29.35	86.43	1.86	0.032	2.56	10.27	37.80	40.82	91.45	3.00	0.052

584	1	1.61	16.01	35.23	28.88	81.73	2.08	0.040	2.03	9.75	39.39	40.76	91.93	3.19	0.052
	2	2.19	14.02	36.36	28.94	81.51	1.98	0.036	2.11	7.83	41.41	43.05	94.40	2.49	0.048
599	1	2.17	17.70	37.98	34.39	92.24	2.17	0.068	2.98	13.69	35.80	41.91	94.38	3.08	0.064
	2	2.28	15.34	39.98	38.92	96.52	2.24	0.044	2.67	12.72	36.50	43.10	94.99	3.31	0.072
607	1	2.26	17.27	36.72	29.78	86.03	2.01	0.040	2.08	10.85	34.24	36.57	83.74	2.98	0.048
	2	2.30	8.55	39.99	31.75	82.59	2.01	0.028	2.54	8.23	36.24	39.28	86.29	3.06	0.044
614	1	1.97	18.62	36.36	29.04	85.99	1.49	0.020	2.87	13.59	39.29	39.39	95.14	1.74	0.104
	2	2.10	14.36	39.33	34.99	90.78	1.88	0.024	2.94	11.81	40.79	38.89	94.43	2.52	0.068
621	1	2.32	19.40	36.63	32.11	90.46	2.49	0.048	2.45	11.46	35.78	42.27	91.96	3.28	0.068
	2	2.40	9.47	36.83	43.29	91.99	2.65	0.048	3.27	7.66	36.58	47.71	95.22	3.41	0.052
628	1	1.50	10.62	35.38	33.51	81.01	2.89	0.044	1.55	10.13	33.49	40.72	85.89	2.94	0.060
	2	1.58	7.29	37.79	35.58	82.24	2.73	0.044	2.21	3.87	35.43	55.52	97.03	2.92	0.064
636	1	2.00	23.02	38.58	29.34	92.94	3.31	0.032	1.80	11.19	37.15	35.73	85.87	2.73	0.080
	2	2.06	17.19	40.17	33.84	93.28	2.68	0.040	2.61	10.67	41.29	43.24	97.81	2.65	0.064
644	1	1.86	20.09	37.98	28.94	88.87	3.42	0.040	2.60	11.80	34.10	36.17	84.67	2.95	0.080
	2	3.49	17.04	39.39	36.90	96.82	3.07	0.048	3.88	8.76	36.60	39.49	87.85	3.11	0.076
652	1	3.05	18.65	34.10	31.85	87.65	1.39	0.064	3.07	11.21	36.05	35.37	85.70	1.38	0.032
	2	3.17	12.98	34.87	36.72	87.74	1.72	0.060	3.90	10.75	39.37	43.84	97.86	2.13	0.044
660	1	1.99	19.11	37.91	29.75	88.76	2.03	0.044	1.99	12.26	34.68	34.11	83.04	3.95	0.076
	2	3.69	13.94	42.27	34.12	94.02	1.88	0.032	3.29	9.84	39.08	38.44	90.65	1.70	0.032
668	1	2.71	18.97	37.28	28.22	87.18	3.15	0.048	3.08	15.95	34.07	38.70	91.80	2.98	0.104
	2	3.35	14.48	38.99	29.68	86.50	3.03	0.048	3.99	13.85	40.24	41.02	99.10	2.29	0.048
Average....	1	2.15	16.96	36.82	31.79	87.72	2.18	0.040	2.35	11.64	35.57	40.37	89.93	2.81	0.068
	2	2.62	12.53	38.97	34.83	88.95	2.14	0.037	2.90	8.63	38.36	44.79	94.68	2.70	0.056

* Determined by subtracting the percentage of CO_2 from the total carbon content and multiplying the difference by the factor 1.724 (44).
† Benzene—alcohol extract.
‡ Includes hemicellulose.

corded in table 2, is less than that calculated by subtracting the percentage of carbonate carbon from total carbon, there is little reason to believe any appreciable quantity of ferrous iron is present. This information shows that different forms of iron and manganese are not the important factors affecting color in these soils.

Some of the coloring in Randall clay may be due to the extra moisture on the fine particles, since the air-dried samples contained more hygroscopic moisture than that of the upland soils, and the oven-dried material appeared darker in color when exposed to atmospheric conditions. When the chemical composition of the organic matter in the upland soil was compared with the analyses of lake bed soils, the latter surface contained an average of 9.30 per cent more waxes and gums, 26.99 per cent more lignin, 31.37 per cent less cellulose, and 3.39 per cent less protein than did the former. According to these data the greatest difference in the composition of these soils is in the "humus" content, which has been described (36) as possessing a definite affinity for water and having a dark brown to black color. These data show that the color in these fine-textured soils formed on lacustrine deposits is more closely associated with the character of organic matter present than with the quantity of total organic matter, nitrogen, or minerals in the parent material or with the texture.

In general, the dark brown or chocolate-colored well-drained upland surface soils contained higher percentages of the humus-forming constituents than did the reddish brown or light-colored soils, which seems to indicate that the dark color in the high-mineral soils in the southern high plains also might be due not so much to total organic matter as to the character of humus present.

SUMMARY

Composite samples of the first and second foot of soil were collected from Randall clay and from adjacent typical well-drained upland soil in the Panhandle of Oklahoma and Texas in 1936. These soils were analyzed for sand, silt, clay, total nitrogen, organic matter, total carbon, carbonates, and organic carbon, waxes and gums (benzene and alcohol extract), cellulose, protein, lignin, iron, manganese, and hygroscopic moisture.

Randall clay was dark gray or black and contained 29.7 per cent less sand, 41.3 per cent more clay, 27.1 per cent less total nitrogen, 27.7 per cent less organic matter, and more hygroscopic moisture in the surface foot than did the upland soil. It also contained less total carbon, carbonates, and organic carbon than did the latter soil. There was no apparent difference in percentage of inert carbon in the different types of soils.

The carbon-nitrogen ratios in both soils were within the normal range of soils recorded by other investigators. The lignin and protein (humus)-nitrogen ratios were considerably higher in Randall clay, and the cellulose-nitrogen ratios were higher in the upland soil.

Although Randall clay contained more iron and manganese than did the

upland soils, the total percentage was very low, and the data obtained show that these elements were not the important factors affecting the color of the dark gray or black clay soils.

The surface of the clay areas contained an average of 9.30 per cent more waxes and gums, 26.99 per cent more lignin, 31.37 per cent less cellulose, and 3.39 per cent less protein than did the upland soils. The greatest difference in the composition of these soils is in the humus content, which has been described by other investigators as possessing a definite affinity for water and having a dark brown or black color.

Data reported show that the color in these fine-textured soils formed on lacustrine deposits is more closely associated with the character of organic matter present than with the quantity of total organic matter, nitrogen, or minerals in the parent material or with the texture.

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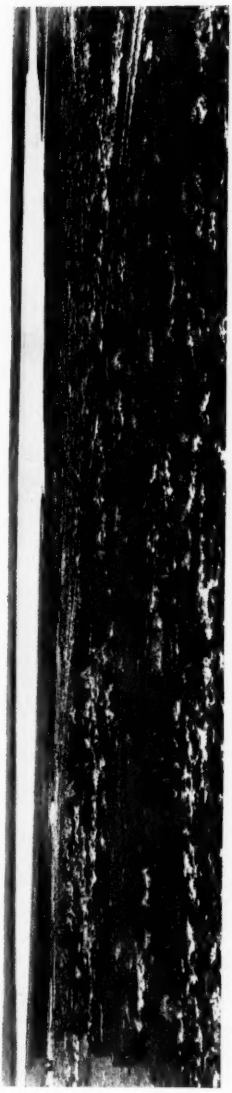
PLATE 1

A TYPICAL VIEW OF A LAKE ON RANDALL CLAY AND OF UPLAND SOIL CONDITIONS AROUND THIS AREA

(Photo made by Soil Conservation Service)

PLATE



EVALUATION OF THE INFLUENCE OF NITROGENOUS FERTILIZERS ON THE ACID-BASE STATUS OF SOILS BY LYSIMETER STUDIES[1]

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The acid or base effects of fertilizers such as sulfate of ammonia or nitrate of soda have been the subject of much discussion in the literature beginning with the paper by Meyer (1) in 1881. Most of these contributions have been reviewed in a recent publication by the senior author (2).

A definite theoretical basis for the evaluation of the acidity or basicity of nitrogenous fertilizers was first developed by Pierre (3). He (4) also proposed laboratory methods for measuring the degree of acidity or basicity of fertilizer materials. Much attention has thus been attracted, and in some states all fertilizers sold must be accompanied by a statement of their expected effects upon the soil, in terms of equivalent calcium carbonate that must be used to neutralize the acidity of the fertilizers or that is replaced by the fertilizers.

In 1929 a series of lysimeter experiments was initiated by the soils department, Connecticut Agricultural Experiment Station, which has yielded data contributing much additional light upon this important subject. Although the work is still in progress, we are now in a position to present the results to date which are pertinent to the problem.

METHOD OF STUDY

The general features of the lysimeter installation are shown in plate 1. The soils are in cylindrical metal containers 20 inches in diameter and of 9-, 20-, and 30-inch depths, depending upon whether surface soil only (7 inches deep), surface soil and upper subsoil, or surface soil and full subsoil were to be studied. The drainage water passing through the soil (designated as *leachate*) is collected in tanks in the interior of the collecting chamber, as shown in figure 2, plate 1.

The leachates have been measured after each period of rainfall sufficient to yield drainage water, and weighted composites for each 6-month period have been subjected to comprehensive chemical analysis. In tanks cropped to tobacco, the crop has been harvested and similarly analyzed. Soils used in these investigations were fully sampled at the beginning of the experiment and again upon removal at the completion of the series. Soil tests for reaction have been made in the spring and late autumn of each year.

[1] Paper presented before American Chemical Society, Rochester, N. Y., September 6–10, 1937.

PLAN OF INVESTIGATION

Series A involved a comparison of the effects of four sources of nitrogen on the uncropped surface layers of four distinctive soil types. The following nitrogenous fertilizers were represented: nitrate of soda, sulfate of ammonia, urea, and cottonseed meal. These were applied in amounts equivalent to 200 pounds of nitrogen an acre yearly, together with other commonly used tobacco fertilizer materials as required to furnish a total of 100 pounds of P_2O_5, 200 pounds of K_2O, and 50 pounds of MgO. The soils were: Merrimac loamy sand (strongly acid), representative of the "shade" tobacco soils of the Connecticut Valley; Merrimac sandy loam (moderately acid), representative of the "Havana seed" tobacco soils; Enfield very fine sandy loam (strongly acid), typical of the "broadleaf" tobacco soils, Wethersfield loam (slightly acid), a common upland soil of the region, used more for dairying than for tobacco. This series was conducted for 5 years, concluding in 1934.

Series B involved a comparison of the effects of 15 sources of nitrogen upon Merrimac sandy loam, with upper subsoil, cropped annually to tobacco without cover crop. The nitrogen sources were as follows: nitrate of soda, nitrate of potash, nitrate of lime; ammonium sulfate, ammonium phosphate; urea, calurea; cyanamid; cottonseed meal, castor pomace, linseed meal; dried blood, tankage, fish meal; stable manure. This series, started in 1929, is still in operation.

Series D replaced series A in 1934. It involves a study of the effects of nitrate of soda, sulfate of ammonia, urea, and cottonseed meal, both with and without adjustment of acid-reacting materials with calcium carbonate. Two lots of Merrimac sandy loam from Windsor field were employed, similar in all respects except that one (soil A) had no previous liming and was moderately acid (pH 5.4) at the beginning of the experiment, and the other (soil B) had been treated twice with dolomitic hydrated lime and was only slightly acid (pH 6.3) when placed in the tanks. This series will be continued until 1939.

RESULTS

Comparisons of effects on different soils (series A)

In order to obtain a comparative picture of the leaching of various basic and acidic constituents during the course of the experiment, the data have been computed in terms of milliequivalents per 100 gm. of soil (2 mm-sieve) in the tanks. A summation of these results for the 5-year period is shown graphically in figures 1 and 2.

It is evident that the equivalent amounts of basic constituents have been largely determined by the nitrate and sulfates. Bicarbonates of relatively smaller magnitude are inversely proportional to the acid tendency of the nitrogen source.

The relative proportion of the various bases appearing in the leachate from the different treatments is markedly different on the various soils. Cal-

cium is of much greater magnitude on the two less acid soils, especially on Wethersfield loam, containing 3.5 m.e. of exchangeable calcium per 100 gm. of soil at the beginning of the experiment, than on the two more acid soils. Since the two more acid soils initially contained only 0.87 and 0.41 m.e. of

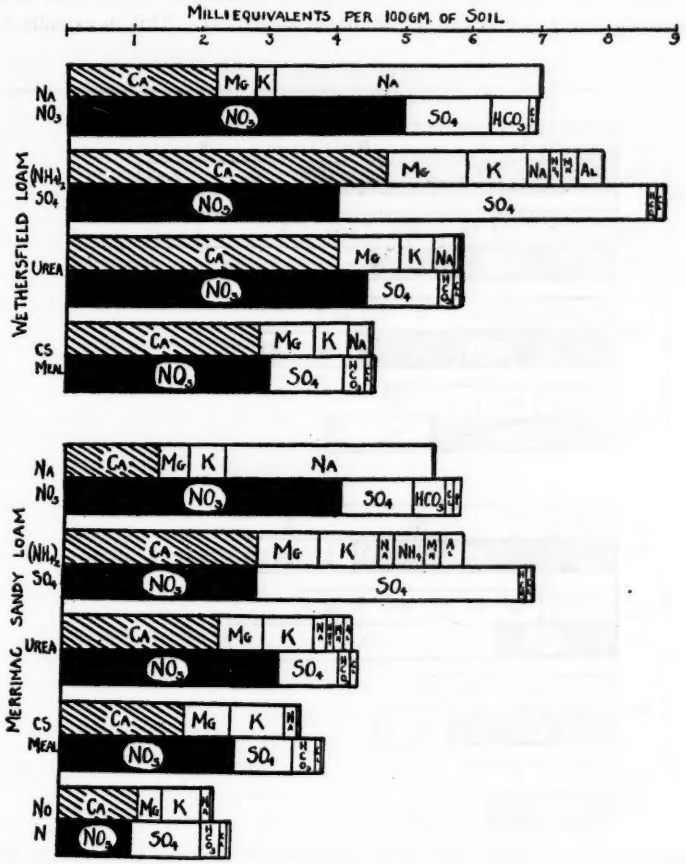

FIG. 1. BALANCE OF BASIC AND ACIDIC CONSTITUENTS REMOVED IN DRAINAGE WATER DURING 5-YEAR PERIOD FROM WETHERSFIELD LOAM AND MERRIMAC SANDY LOAM, WINDSOR LYSIMETERS, SERIES A

exchangeable Ca, respectively, the possibilities of calcium loss are much less. On corresponding treatments potassium is proportionately greatest on Merrimac sandy loam, with the highest initial exchangeable potassium (0.39 m.e. per 100 gm.). Magnesium, applied in the fertilizer treatment, has been leached to a marked degree, especially under the sulfate of ammonia treatment.

It is of special interest to note that the relative amounts of aluminum and manganese removed from the more acid soils were much larger than the amounts removed from the less acid soils, and the amounts of each element removed were proportional to the degree of acidity of the nitrogen source. It is also to be recognized that the excess of anions over determined cations is greatest under the sulfate of ammonia treatment. This is explained, at

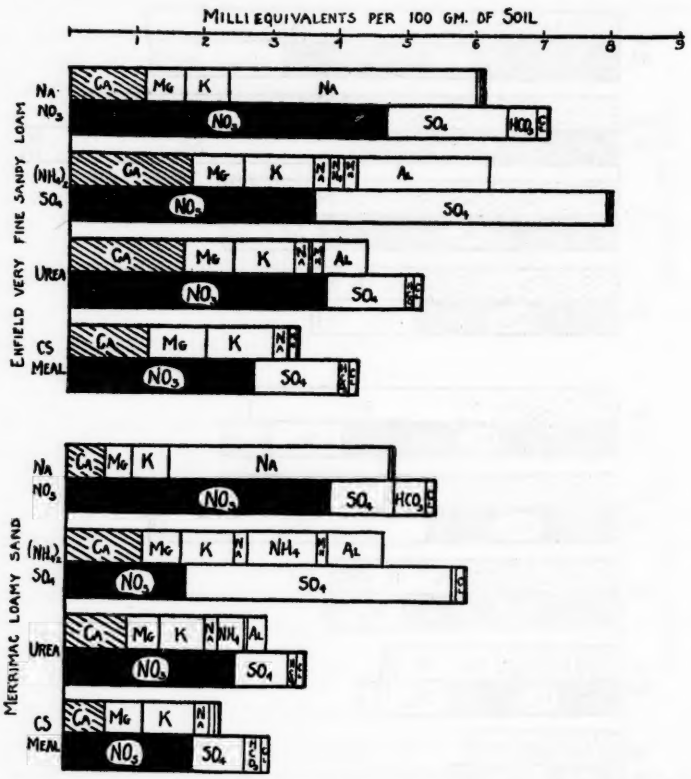

FIG. 2. BALANCE OF BASIC AND ACIDIC CONSTITUENTS REMOVED IN DRAINAGE WATER DURING 5-YEAR PERIOD FROM ENFIELD VERY FINE SANDY LOAM AND MERRIMAC LOAMY SAND, WINDSOR LYSIMETERS, SERIES A

least in part, by zinc dissolved by acid-reacting constituents of the soil solution from the galvanized screens of hardward cloth that had been placed to separate the sand filter bed from the superimposed soil. When these screens were removed at the conclusion of the experiment, the original protective coating of asphaltum paint had been partially lost, and corrosion was directly related to the degree of acidity of the soil in the tank. It is felt, however,

that the zinc largely replaced aluminum, manganese, and iron, rather than the normal soil bases, such as calcium, magnesium, and potassium.

The data supplied by this experiment (table 1) enable one to calculate from intake (applied in treatment) and output (leached) the theoretical gain or loss of basic constituents. This may then be compared with the actual change in the exchangeable base status of the soil and with the final pH attained at the end of 5 years. For brevity, data on individual bases are not included, but these are presented in a previous publication (2).

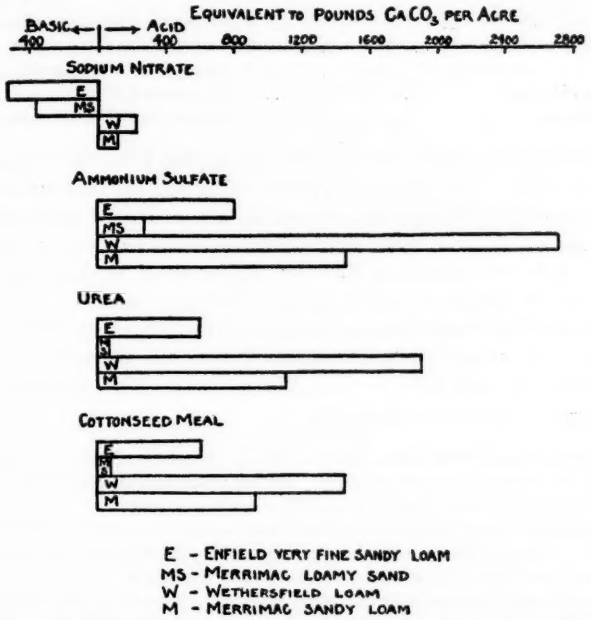

FIG. 3. AVERAGE EFFECT OF NITROGENOUS FERTILIZERS AS MEASURED BY THE BASE STATUS OF THE SOIL AT THE END OF 5 YEARS OF ANNUAL TREATMENT WITH 200 POUNDS OF NITROGEN, WINDSOR LYSIMETER SOILS, SERIES A

The data presented in table 1 show strikingly the differences in the operation of a given nitrogen source on various soils, depending on their initial base status. The discrepancies between net change as calculated from leaching and additions and the actual change in exchangeable bases involve both fixation of added constituents in nonexchangeable form and liberation from soil material not normally entering into base-exchange reactions. Thus, calcium is leached to a greater degree than could be predicted from its loss in the exchangeable form, especially under sulfate of ammonia treatment. On the other hand, both added potassium and sodium are apparently "fixed" to a significant degree.

In order to present the data in a form that may be interpretable from the standpoint of a liming material that may be needed to offset the acid tendency of the fertilizer, certain results have been recalculated in terms of acid or basic effects, in terms of equivalent pounds of calcium carbonate per acre of surface soil. Figure 3 shows the effects of each treatment upon the actual base-exchange status of the four soils. These are to be compared with the calculated theoretical effects, based on Pierre's method. They are as follows, for the various treatments during the 5-year period:

Nitrate of soda	1,368 pounds $CaCO_3$ per acre, basic
Sulfate of ammonia	5,775 pounds $CaCO_3$ per acre, acid
Urea	2,203 pounds $CaCO_3$ per acre, acid
Cottonseed meal	2,525 pounds $CaCO_3$ per acre, acid
No nitrogen	1,368 pounds $CaCO_3$ per acre, acid

Since no crop is involved, the total nitrogen applied is considered as exerting its full acid effect. The other constituents furnished a net theoretical basicity of 1,368 pounds $CaCO_3$ per acre, except on the cottonseed meal treatment. In this case the material other than cottonseed meal required to supply 100 pounds of P_2O_5, 200 pounds of K_2O, and 50 pounds of MgO per acre yearly resulted in a net basicity of only 253 pounds $CaCO_3$ per acre.

A comparison of these values with those given in figure 3 shows that the soil changes are of much smaller magnitude than the theoretical, especially on the more acid soils with low initial base status. The following compensating factors, however, are involved:

The nitrogen added is not fully nitrified. This is especially true for cottonseed meal, which has yielded only 65 per cent recovery. Neither urea nor sulfate of ammonia was fully active either, recovery being 90 per cent and 88 per cent respectively.

Added sulfates from sulfate of ammonia still remain in the soil, as calcium sulfate or similar compounds. Sulfate recovery in the leachings from this treatment has been incomplete to the extent of from 558 to 1,025 pounds of equivalent $CaCO_3$ per acre.

Portions of the nitrates and sulfates leached have been combined with aluminum, manganese, and other undetermined bases, such as zinc as a contaminating factor. This is conspicuously true under the sulfate of ammonia treatments, particularly on the more acid soils of low initial base status.

Bicarbonate tends to disappear as an anion effective in base leaching under acid-reacting fertilizer.

Bases such as calcium and magnesium, not normally exchangeable in the soil, are liberated by the treatment and become leached; conversely some potassium and sodium added in the treatment are fixed and lose their effectiveness in determining the reactive status of the soil.

Nitrate of soda tends toward a higher soil pH than would normally be expected at a given degree of base saturation. This is apparently associated with a trend toward abnormal proportions of monovalent cations in the base exchange complex.

Effects on a soil cropped to tobacco

Series B has involved the removal of constituents in both crop and leachate. The latter is consequently of smaller magnitude than that in series A. Data for this series for the 1929–1934 period are presented in table 2.

TABLE 1

Comparison of net change in base status of soil from treatment and leachings, and change in exchangeable bases*, in milliequivalents per 100 gm. of soil*

(Series A)

	NET CHANGE FROM LEACHING DATA	INITIAL EXCHANGEABLE BASES	CHANGE IN EXCHANGEABLE BASES	INITIAL pH	RESULTANT pH
Wethersfield loam		*4.62*		*5.44*	
Nitrate of soda	0.93+		0.46−		6.22
Sulfate of ammonia	3.69−		3.54−		4.15
Urea	2.32−		2.57−		4.89
Cottonseed meal	1.91−		2.07−		5.03
Merrimac sandy loam		*2.25*		*5.17*	
Nitrate of soda	0.79+		0.27−		5.89
Sulfate of ammonia	2.19−		1.55−		4.12
Urea	1.24−		1.16−		4.70
Cottonseed meal	1.48−		1.16−		4.83
No nitrogen	0.44+		0.09−		5.52
Enfield very fine sandy loam		*1.48*		*4.70*	
Nitrate of soda	1.84+		0.42+		5.34
Sulfate of ammonia	0.52−		0.76−		4.03
Urea	0.18−		0.40−		4.45
Cottonseed meal	0.72−		0.44−		4.49
Merrimac loamy sand		*0.80*		*4.99*	
Nitrate of soda	1.23+		0.54+		5.98
Sulfate of ammonia	0.07+		0.25−		4.64
Urea	0.50+		0.13−		5.01
Cottonseed meal	0.15+		0.16−		4.99

* Including calcium, magnesium, potassium, and sodium.

TABLE 2

Comparisons of net changes in base status of soil from treatment, crop removal and leaching, and changes in exchangeable bases*, in milliequivalents per 100 gm. of surface soil*

(Series B—1929–1934)

	NET CHANGE FROM LEACHING AND CROP REMOVAL	INITIAL EXCHANGEABLE BASES	CHANGE IN EXCHANGEABLE BASES	INITIAL pH	RESULTANT pH (MAY, 1934)
Merrimac sandy loam		*2.25*		*5.17*	
Nitrate of soda	0.96+		0.39+		6.33
Sulfate of ammonia	2.81−		1.77−		4.01
Urea	0.99−		0.97−		4.90
Cottonseed meal	1.46−		0.80−		4.97
No nitrogen	0.47+		0.02+		5.69

* Including calcium, magnesium, potassium, and sodium.

From a comparison of table 2 with table 1, for uncropped Merrimac sandy loam, it appears that the nitrate of soda treatment has been somewhat more alkaline and the sulfate of ammonia more acid in their effects, as measured both by net gains or losses by leaching and crop removal and by soil change. No measurements of the effect upon the partial subsoil in the uncropped tanks will be possible, however, until the series is completed.

The more alkaline tendency of the nitrate of soda is to be expected, to a much greater degree if we are to accept Pierre's assumption of nitrogen assimilated by the crop in combination with only 50 per cent of its base-equivalence. The acid tendency of sulfate of ammonia should thus be only three-fourths

TABLE 3

Soil reactions after 8 years under treatment—Series B

(200 pounds nitrogen per acre annually)

TREATMENT	pH
Cyanamid	7.13
Nitrate of soda	6.97
Nitrate of potash	6.58
Stable manure	6.48
Nitrate of lime	6.07
No nitrogen	6.04
Calurea	5.19
Tankage	4.90
Castor pomace	4.89
Fish meal	4.85
Linseed meal	4.84
Urea	4.83
Cottonseed meal	4.81
Dried blood	4.70
Ammonium phosphate	4.29
Ammonium sulfate	4.02
Initial reaction	5.17

as great as that on the uncropped soil. The actually greater net acid effect is probably explained by the fact that some of the ammonia nitrogen was lost in the leachings from the shallower tanks but not in the deeper cropped ones.

Detailed calculations from crop analyses of tobacco removed from this series indicate that the following percentages of nitrogen could have been taken up without a corresponding amount of a base (silica not determined):

	per cent
Nitrate of soda	26.1
Sulfate of ammonia	36.9
Urea	19.6
Cottonseed meal	23.0
No nitrogen	9.0

It is also to be considered that the crop was removed during only 4 of the 5 years (the first being destroyed by hail) and that the nitrogen was applied in much larger amount than was required to replace that removed by the crop.

Tobacco is a "high-ash" crop, and it is probably not typical of most field crops with respect to its acid-base balance.

Lysimeter data with respect to effects of other nitrogen treatments in this series upon the base status are omitted, to await publication of final data for the entire experiment. Soil reaction trends, however, are in line with results to be expected from their theoretical acidities or basicities, as shown by the pH values in table 3, determined at the end of 8 years.

Adjustment of acid-reacting nitrogen sources

Series D, started in 1934, affords an opportunity for determing the practicability of maintaining the base-status of the soil by adjustment of acid-reacting fertilizers with equivalent amounts of liming materials.

Although series A indicated that the net effect of continued use of a fertilizer could not be quantitatively predicted on the basis of previous calculation, it remained to be proved whether or not liming applied concurrently with the fertilizer in amounts equivalent to the theoretical acidity would accomplish the desired stabilization of the base status of the soil.

As far as reaction is concerned, the results to date are very gratifying. Figures 4 and 5 show annual pH values during the first 3 years of this experiment, both without and with acid adjustment, on the two soils at different initial acidities. It is apparent that the calculated amounts of calcium carbonate have prevented the downward trend otherwise evidenced by all acid-reacting fertilizers. On the other hand, no consistent upward trend has been accomplished by the use of lime carbonate in these comparatively liberal amounts, scaled to the calculated effects of the treatment.

In the foregoing trials the materials used in the "no nitrogen" treatments, supplying 100 pounds of P_2O_5, 200 pounds of K_2O, and 50 pounds of MgO per acre yearly, were formulated so as to be theoretically neutral in effect. Also, calcium was applied in the same quantity in all cases except on the unadjusted sulfate of ammonia treatment. On one set of sulfate of ammonia treatments, this amount of calcium was added as calcium carbonate in order to compensate for the sulfate constituent of the fertilizer, thus placing it on practically the same basis as urea. Sulfate, as calcium sulfate, was used in the same quantity as for sulfate of ammonia on all other treatments. It was necessary to supply a small amount of sodium, as sodium sulfate, in the cottonseed meal treatment.

An interesting feature of the results to date is the difference in relative leaching of the various cations from soil A, unlimed previously, and from soil B taken from a plot that had been limed with dolomitic hydrate. This is shown graphically in figure 6. The greater relative loss of magnesium from soil B and the higher relative loss of potassium from soil A for all treatments are of special significance. Data for the 3 successive years indicate that these

differences become less as the excess of exchangeable magnesium in soil B over soil A is depleted. Sulfate of ammonia, without adjustment, is rapidly bringing these two soils to the same low base status, although they were initially very dissimilar. The use of calcium carbonate in amounts sufficient to correct the theoretical acidity of the material is maintaining a fairly constant total base status, as indicated by pH trends, but the proportion of calcium to other bases is being much more markedly increased on soil B than on soil A. If

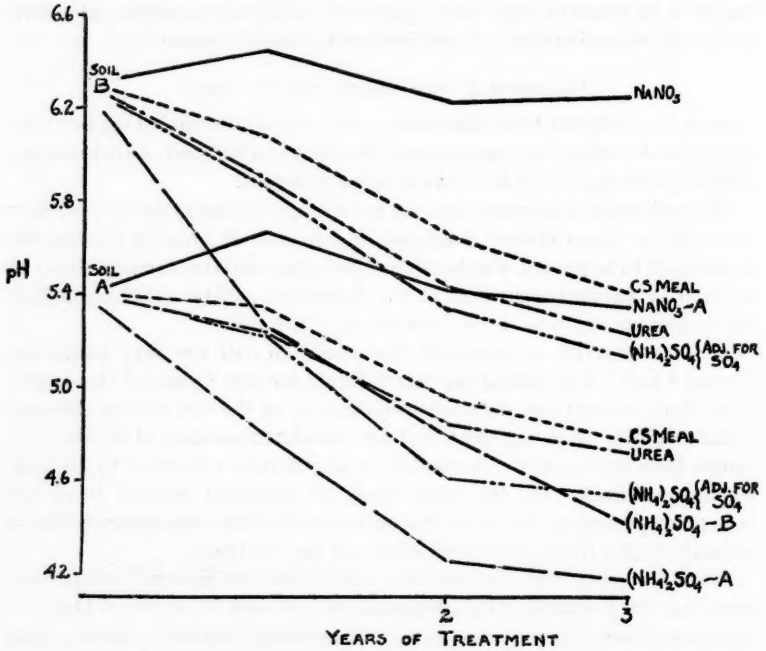

FIG. 4. YEARLY TRENDS OF REACTION, SOILS IN WINDSOR LYSIMETERS, SERIES D, WITHOUT ACID ADJUSTMENT

dolomite, however, had been used to correct the acid tendency of the fertilizer, the converse might have occurred.

No soil determinations except pH have been made on series D. The lysimeter data have been analyzed, however, in order to show net losses or gains in base status during the 3 years, computed in terms of calcium carbonate equivalence per acre.

These results are presented in table 4.

As in series A, the soil with the higher initial base status has been more severely depleted by the fully acid sulfate of ammonia treatments. On the

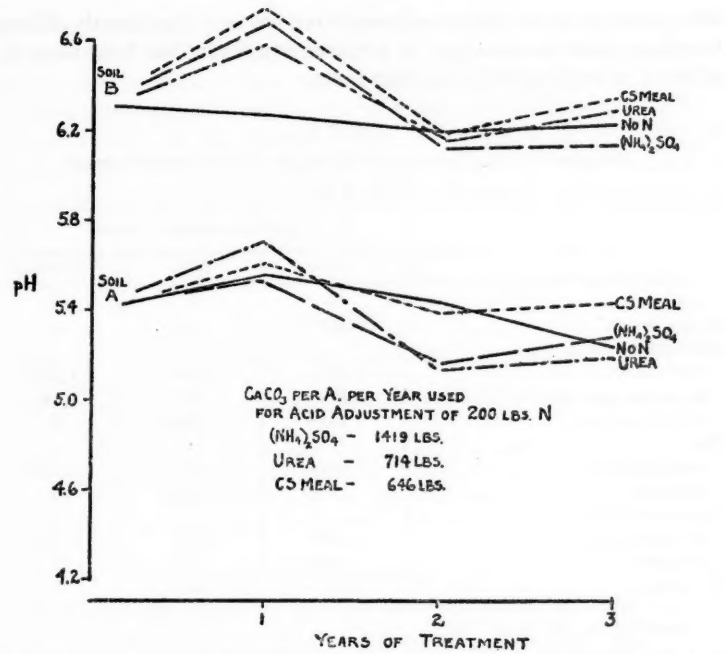

FIG. 5. YEARLY TRENDS OF REACTION, SOILS IN WINDSOR LYSIMETERS, SERIES D, WITH ACID ADJUSTMENT

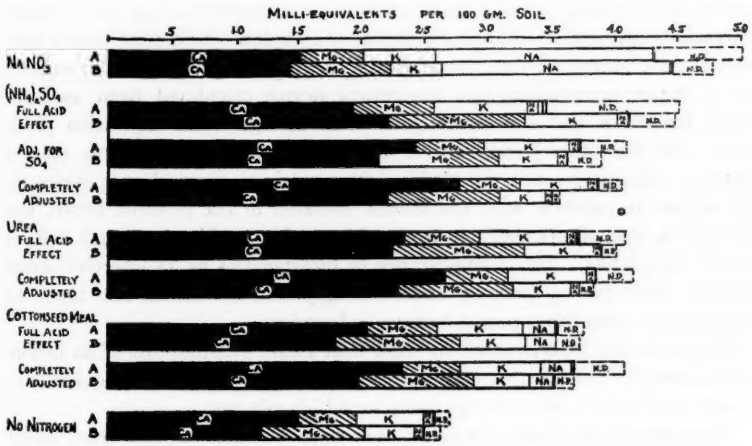

FIG. 6. BASIC CONSTITUENTS LEACHED FROM SOILS A AND B, WINDSOR LYSIMETERS, SERIES D, 1934–1937

other hand, urea and cottonseed meal have not been significantly different. It appears that the lime used in fertilizer adjustment has been more than sufficient to compensate for leaching losses.

TABLE 4

Net gains or losses of base status during first 3 years under treatment*

(Series D)

	$CaCO_3$ EQUIVALENCE, PER ACRE		
	Soil A	Soil B	Theoretical
	lbs.	*lbs.*	*lbs.*
Nitrate of soda	691−	864−	0
Sulfate of ammonia			
Full acid effect	2,857−	3,675−	4,257−
SO_4 adjustment only	1,889−	1,797−	2,196−
Full adjustment	133+	530+	0
Urea			
No adjustment	1,889−	2,131−	2,142−
Adjusted	81+	230+	0
Cottonseed meal			
No adjustment	1,348−	1,336−	1,938−
Adjusted	438+	611+	0
No nitrogen	495−	518−	0

* Including Ca, Mg, K, and Na.

SUMMARY

The results obtained in these studies have shown that acid-reacting fertilizers, by increasing losses of basic constituents through leaching, tend toward increased unsaturation of the base-exchange complex. Soils that have a high initial base status become depleted of bases as a result of the treatment, to a degree approaching the theoretical degree calculated from assumptions based on the stoichiometry of their biological decomposition products. On the other hand, soils of low exchangeable base content cannot supply sufficient amounts of readily active calcium, magnesium, potassium, or sodium to combine with the anions liberated in the process; hence, the change in base status resulting from acid-reacting fertilizers on such soils is much diminished. The disappearance of bicarbonates as an effective anion in base depletion by leaching under strongly acid conditions also tends to produce less than the expected increase in base loss.

Results under tobacco cropping show that Pierre's assumption of an intake of nitrogen by the tobacco crop corresponding to twice its base equivalence is not applicable in all cases, especially for a high-ash crop.

Experiments in adjustment of acid-reacting fertilizers indicate that, under uncropped conditions, the use of calcium carbonate in amounts equivalent

to the theoretical acidity of the fertilizer tends to stabilize the soil at approximately its initial pH. Leaching data to date in this uncompleted experiment, however, seem to indicate that calcium has been added in greater amounts than have been required to replace the equivalent base leaching.

Nitrate of soda has had a greater effect in raising the pH than could be explained from its effect upon total base saturation. This is considered to be due to the increase in proportion of monovalent cations in the base-exchange complex.

Incomplete results with several other nitrogenous materials give data indicating effects commensurate with the character of the nitrogen source.

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PLATE 1

WINDSOR LYSIMETERS

FIG. 1. Exterior view
FIG. 2. Interior of collecting chamber

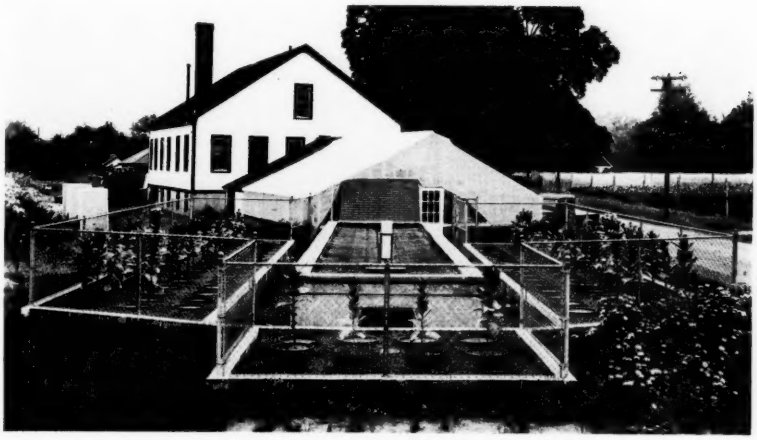

FIG. 1

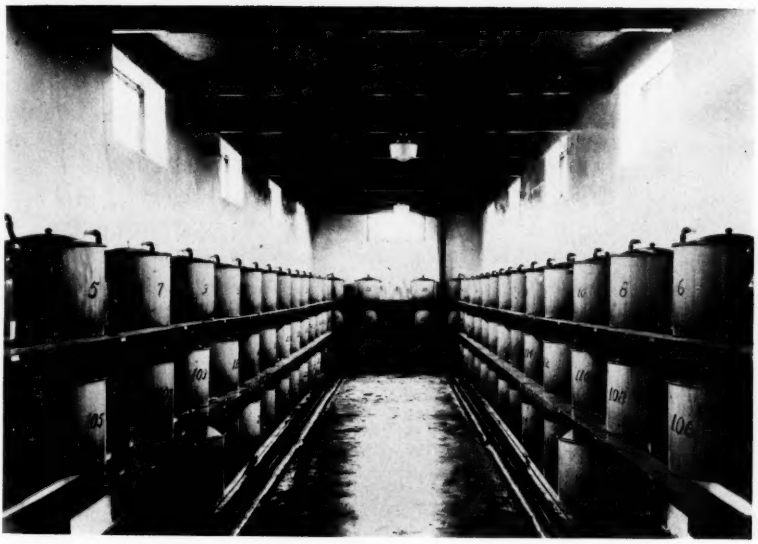

FIG. 2

UNIV. OF MICH.

NUTRITION STUDIES WITH CORN: II. A STATISTICAL INTERPRETATION OF THE RELATION BETWEEN THE IONIC CONCENTRATION OF THE CULTURE SOLUTIONS AND THE ELEMENT CONTENT OF THE TISSUES[1]

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The present paper is the second of two publications dealing with nutrition studies with corn. In the previous publication (1) an attempt was made to isolate and deal statistically with the effects of individual ions upon growth in artificial cultures. The data considered in the following pages were obtained in connection with the previous study and deal statistically with the relations between the concentration of each ion in the nutrient solutions and the content of the corresponding elements in the sap of the plants and the elemental content of the tissues as a whole. Since the experimental setup, the calculation of the solutions in the variable ion proportion series employed, a thorough description of the materials used, and a detailed discussion of the methods employed in these studies have been presented in the previous publication (1), they will be omitted from the present paper. The methods used in the chemical analyses of the plant material will be presented but will not be discussed in detail except in those cases where necessary modifications resulted in pronounced deviation from the standard methods.

The development of the aforementioned variable ion proportion series has provided a means of studying, by a different method of approach, the effect of individual ions upon metabolic activity as expressed by growth measurements. The purpose of this work is to determine, by a similar method of approach, the effect of variations in the concentration of each ion in the nutrient solution upon the content of the various elements in the plant sap and in the tissue as a whole. It is a well-known fact that ions will accumulate in plant cells against an apparently steep concentration gradient. Furthermore, the absorption of any one ion from a mixture of ions in a nutrient solution and the accumulation[2] of the corresponding element within the cells of the plant, is a function of the concentration of the ion in the external medium.

It is also well known that the rate of penetration of the various ions is

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of plant physiology.

[2] As used in connection with the data presented in this paper the term "accumulation" denotes the tissue content at harvest of the elements under consideration.

related to their mobility, and that the mobility, in water solution, is in turn a function of the degree of hydration of the ion. Arranged in series according to the rates of penetration, the three cations to be dealt with are listed by Seifriz (15) as follows:

$$K^{+} > Mg^{++} > Ca^{++}$$

The anions rank in the following order:

$$NO_3^{-} > HPO_4^{-} > H_2PO_4^{-} > SO_4^{-}$$

The comparison of the anions of the monobasic and diabasic phosphate salts are taken from velocities as calculated from conductance data by Buehrer (4).

The rate of penetration of an ion, in itself, would not determine the ultimate internal concentration of the ion, however, were it not for the capacity of the colloidal protoplasmic complex to hold ions. McGeorge (10) postulates a chemically equivalent base exchange system in plants, which in conjunction with the Donnan equilibrium and the amphoteric properties of proteins probably accounts for the apparent accumulation gradient.

In an attempt to simplify consideration of the whole problem of accumulation of ions, Brooks (3) and Hoagland et al. (8) have based several studies on the absorption of ions by large one-celled plants. Despite the valid criticism by Steward (17) that these large mature cells are not strictly comparable to the immature absorbing cells of roots, Hoagland et al. have reached some important conclusions. They state that "the rate of accumulation of the anion was conditioned primarily by the presence in suitable concentration of a cation capable of ready penetration and accumulation rather than upon alterations in the protoplasm resulting from different proportions of mono- and di-valent ions." This present study also assumes, and it is believed with justification, that within the limits of the various ionic concentrations employed in these experiments, the rate of penetration of the ions is not significantly influenced by the nature of the membrane. For this reason antagonism, in its commonly accepted sense, will not be considered here. Hoagland et al. have data which support their conclusions that there is an active competitive effect in ionic penetration. They suggest the possibility of one cation's decreasing the rate of accumulation of another from the standpoint that the cations compete because of the similarity of the electric charge. They also postulate a definite competition between anions, the more active ion repressing the accumulation of the less active. Between ions of opposite charge an acceleration of the penetration and accumulation of a slowly moving cation by a rapidly moving anion would complete the hypothesis for all possible nutrient ion proportions.

With respect to higher plants, Breazeale (2) states that the presence of nitrate increases markedly the absorption of potassium in wheat seedlings. Hoagland and Broyer (7), from work with excised roots, conclude that the

accumulation of both anions and cations is associated with active aerobic respiration of the roots. Steward (17) maintains that the correlation with respiratory rates is an indirect effect, and that the more direct correlation is with metabolic activity. Jacobson and Swanback (9) found that when nitrogen is supplied as nitrate there is a pronounced increase in calcium absorption over that which occurs when nitrogen is supplied as ammonium.

In this work it was considered that, with the method devised for analyzing the effect of concentration of each ion in the solution upon accumulation of an element corresponding to any other ion within the plant, a measure of interionic relationships might be established without regard to the complexity of the plant used. Obviously, however, different species may give different results, because of differences in metabolic mechanism.

METHODS USED IN THE ANALYSES

The plant tissues were divided into two fractions, the stems and the leaves. The stem fraction included the entire stem, leaf sheaths, and all other tissue below the uppermost visible blade-sheath junction. This fraction was, therefore, somewhat heterogeneous with respect to the nature of included tissues. The leaf tissue consists of blades only, including the midrib.

The methods used in the analyses of the elements were standard methods, selected with regard to convenience, equipment, and the amount of sample available. All analytical data except those for nitrogen were obtained from aliquots of the same digested salt solutions. For convenience in discussion, the data will be referred to as "soluble" and "total," although the "soluble" fraction undoubtedly does not include all water-extractable material and probably includes a very small amount of insoluble material in colloidal form not filterable by the methods used.

The "total" determinations were obtained from tissue digested on a steam bath with aqua regia, alternating with fuming nitric acid. The crystalline salt mixture was then taken up with a small amount of concentrated HCl and dried again to eliminate the color of NO_2, after which it yielded a clear, colorless solution when taken up with 5 per cent HCl. Aliquots of this solution were then used in the various determinations. Total N was determined separately by Ranker's (12) modification of the Kjeldahl procedure.

The data on "soluble" extracts were determined from plant juice expressed from frozen tissue and filtered by suction through three thicknesses of filter paper (Whatman #4, #1, and #50). Aliquots of this sap were then dried on a steam bath and digested as was the tissue for "total." The determination of total soluble N was made on a hot water extraction of frozen tissue, again according to Ranker's modification of the Kjeldahl procedure.

Calcium was precipitated as the oxalate, filtered, washed, and then taken through the filter with hot 10 per cent H_2SO_4. This was then titrated with 0.05 *N* permanganate at 70°C. in the standard volumetric procedure (18).

Magnesium was determined on the filtrate from the calcium determinations.

The sensitive oxine precipitation method of Redmond and Bright (13), with a final titration with standard thiosulfate, was used.

Phosphorus was determined colorimetrically as the phosphate by the revised Deniges method of Truog and Meyer (19), using a Klett biocolorimeter.

Sulfur was determined gravimetrically as the sulfate by precipitation with barium chloride, under standard conditions (14).

Potassium was determined colorimetrically after precipitation as the chloroplatinate, using the pink color developed upon the addition of potassium iodide (5).

TABLE 1

A summary of the mineral analyses of leaf tissue, expressed in milligrams per 100 gm. fresh tissue

The treatment numbers are arranged in descending order of the average fresh weights of the plants.

TREATMENT NUMBER	AVERAGE WEIGHT PER PLANT	NITROGEN		POTASSIUM		CALCIUM		MAGNESIUM		PHOSPHORUS		SULFUR
		Soluble	Total	Soluble	Total	Soluble	Total	Soluble	Total	Soluble	Total	Soluble
	gm.											
11	428	92.3	354	467	492	20.5	40.0	26.0	28.9	28.6	34.9	17.2
3	407	135.6	441	243	269	32.1	57.4	65.3	69.9	29.2	36.3	15.3
1	310	75.3	324	260	337	25.6	43.6	49.8	54.9	35.0	41.0	39.8
5	297	142.2	470	148	205	68.9	125.4	54.7	69.4	25.7	33.0	13.1
10	277	64.5	332	380	460	16.1	29.2	10.0	12.0	53.8	59.1	41.0
6	269	88.9	399	140	207	42.0	87.6	43.7	66.4	41.1	48.3	23.2
4	175	44.8	252	428	450	16.1	32.7	26.2	26.6	33.9	35.5	53.6
15	173	160.0	435	195	245	18.1	21.7	107.0	145.2	35.3	41.3	14.3
2	172	57.3	261	327	384	24.8	40.0	29.3	30.9	82.5	91.7	25.8
7	161	57.7	276	180	224	38.3	86.8	25.0	32.8	22.4	32.4	38.0
12	159	50.7	257	346	423	11.4	20.8	10.7	11.9	31.0	36.3	55.8
14	158	155.5	418	218	240	10.4	20.3	87.0	97.5	74.8	76.9	17.0
9	150	45.5	244	363	452	13.9	27.6	15.7	16.8	101.8	106.7	28.8
8	130	51.6	286	259	294	39.9	81.7	24.3	31.8	89.2	100.2	22.8
16	129	61.6	300	170	261	9.5	19.0	72.6	105.3	93.7	107.2	25.0
13	112	62.9	291	174	293	9.4	22.1	70.5	99.7	34.3	36.1	75.7

Each of volumetric and gravimetric determinations was the average of duplicate determinations, and each of the colorimetric determinations was the average of triplicate determinations.

All the methods were carefully checked by the use of a synthetic salt solution composed of standard salts plus traces of iron, manganese, and boric acid. The results proved the volumetric and gravimetric methods accurate to within 1 per cent relative error, and the colorimetric methods to within 4 per cent error.

PRESENTATION OF DATA

The data representing the concentration of each of the elements studied are presented in tables 1 and 2. The importance of variations in content of

nutrient elements in plant tissue, particularly in the soluble fractions, cannot be adequately judged unless the connection with water is thoroughly understood, inasmuch as these elements are physically and chemically active only in solution. For this reason the data are presented on a fresh-weight basis.

A brief consideration of tables 1 and 2 reveals the impossibility of any attempt to interpret the metabolic status of the plants without simultaneous consideration of all the elements involved. For example, a deficiency in content of nitrogen within the tissues results in a dwarfed plant and indirectly in a proportionate percentage increase in accumulation of other elements. Unless such accumulation, therefore, is interpreted simultaneously with

TABLE 2

A summary of the mineral analyses of stem tissue, expressed in milligrams per 100 gm. fresh tissue

The treatment numbers are arranged in descending order of the average fresh weights of the plants.

TREATMENT NUMBER	AVERAGE WEIGHT PER PLANT	NITROGEN		POTASSIUM		CALCIUM		MAGNESIUM		PHOSPHORUS		SULFUR
		Soluble	Total	Soluble	Total	Soluble	Total	Soluble	Total	Soluble	Total	Soluble
	gm.											
11	428	91.6	141.0	320	380	9.2	12.3	16.8	19.7	20.1	21.9	5.8
3	407	132.0	171.8	176	187	19.4	24.9	35.7	58.0	16.2	18.7	5.4
1	310	50.4	91.8	162	255	10.8	12.7	20.9	37.0	30.7	31.4	12.9
5	297	126.0	177.0	77	78	46.7	60.5	40.9	46.4	14.2	15.8	5.1
10	277	37.6	78.1	390	548	5.5	6.6	6.9	9.1	36.0	37.3	15.1
6	269	58.0	114.2	51	52	26.6	31.3	36.6	40.6	30.5	31.1	9.0
4	175	29.4	65.6	287	507	4.9	6.4	10.0	13.7	23.4	28.1	14.7
15	173	158.2	201.0	145	151	5.4	7.3	79.5	96.5	23.5	25.8	5.6
2	172	27.8	72.3	192	328	9.5	17.0	15.2	19.0	34.3	48.0	8.2
7	161	30.0	77.6	135	141	34.0	42.6	26.7	33.4	24.1	27.3	14.5
12	159	26.4	70.2	259	477	3.0	3.3	4.9	6.7	25.1	26.9	18.9
14	158	123.0	183.8	124	174	4.4	6.7	57.5	82.3	30.7	37.4	5.4
9	150	25.2	68.0	414	482	5.7	6.2	10.2	13.5	60.8	65.3	13.9
8	130	29.0	92.8	169	192	32.2	43.0	26.0	34.4	58.5	62.6	9.8
16	129	38.6	85.3	156	258	3.9	5.1	54.4	64.0	60.2	64.6	11.1
13	112	39.5	96.8	212	256	2.5	6.3	38.5	59.5	21.6	30.0	20.8

respect to the nitrogen content of the tissue, the study is valueless. Similarly, absorption and accumulation of magnesium by plants grown in solutions high in magnesium result in a toxic condition, and these plants also fail to make rapid growth. Tissue analyses of such plants as those in treatment 15 show a relatively high nitrogen content. Analysis of the data of this treatment is not significant, then, without consideration of the effects of the very high concentration of magnesium. Magnesium toxicity is not primarily the result of a deficiency of calcium or potassium in the tissues, but rather is associated with an interference with the usual course of metabolic activities of the plant.

The method of successive approximations as applied to curvilinear correlations was employed with these data in an attempt to discover the effect of the several independent variables (the concentrations of the various ions in the substrate) upon the dependent variable (the concentration of any one element in the tissue). This is the same method which was used to interpret growth relationships in the previous paper (1) already referred to. The regression equation upon which the computations were based is stated as follows:

$$X_1 = a' + f(X_2) + f(X_3) + f(X_4) + f(X_5) + f(X_6) + f(X_7)$$

In this equation, the dependent variable, X_1 represents the milligrams of the element under consideration per 100 gm. of fresh plant material. The factor a' is a constant, ordinarily included in the value determined for $f(X_2)$. X_2, an independent variable, represents the nutrient solution concentration of the element under consideration and is expressed in terms of the relative partial osmotic concentration produced by that ion in the substrate (1). X_3 to X_7, all independent variables, are arranged in order according to decreasing mobility of the ions, but alternating with respect to charge. The one exception to this order is the interchange of calcium and magnesium, contrary to their ionic mobilities. This was done because of the apparently greater relative importance of calcium over magnesium in the growth responses of the plants here employed.

Calculation of the coefficients of part correlation was omitted because it was considered that their inclusion here was not particularly important. However, the Z' values, which represent the residuals not accounted for in the analysis, are included in tables 3 to 13. These residuals are low enough to make the more significant net regression curves reliable statistically. Included in tables 3 to 13 are the analytical data of the various elements, presented in regular order with reference to the treatment numbers, as well as the X' values. The X' values may be directly substituted in the regression equation in place of the corresponding $f(X)$ terms.

For any one table of this group, these X' terms represent the total statistical significance of the ion designated, in relation to the analysis value of the element found in the tissue. The constant a' is included in the X_2' values, and the other X' values ($X_3' - X_7'$) represent positive or negative corrections for the X_2' values (6).

The net regression curves (figs. 1–6) represent the effect of different concentrations of each ion in the nutrient solution upon the accumulation in the tissue of the element under consideration. The effect of all other ions is statistically removed from each curve.

The use of corn, or any of the other higher plants in this type of experiment, necessarily involves not only tissue differentiation with its accompanying change in plant composition during the course of the growth cycle, but also rather extreme differences in rates of growth between plants under different nutrient treatment.

It may reasonably be expected that under conditions of high growth rates a lower relative percentage content of the several elements involved may result and this, in turn, may result in an overshadowing of the effects of the apparent rates of ion penetration.

A consideration of the hypothesis of accumulation of Hoagland et al., with respect to the plants employed in these experiments, must take into account the total tissue growth in connection with a discussion of figures 1 to 6, and this factor has been considered in the interpretation of the data here presented.

Figure 1 presents evidence in support of Hoagland's hypothesis by the statistical method of interpretation. The curves representing the allowance for magnesium concentration (estimated magnesium in milligrams plotted against a concentration of magnesium in the substrate), include the a' values; all other curves represent plus or minus correction values for the magnesium allowance curves. A more detailed explanation of this type of curve has been previously published (1). Figure 1 is constructed from the X' values presented in tables 3 and 4. The toxic effect of magnesium in high concentration was previously found practically to eliminate growth differences in these plants (1), which might result from the wide variations in concentrations of the other ions, particularly the nitrate ion.

The effect of the other ions upon the accumulation of magnesium in the plant should best illustrate the hypothesis of Hoagland, because little of the magnesium absorbed by the plant is actually assimilated, as is clearly indicated in figure 1 by the slight difference between the values of the total and soluble magnesium contents of both leaf and stem tissues.

It will be observed that the curves illustrating the effect of the concentration of magnesium in the substrate on the accumulation of magnesium in the tissues deviate slightly from the theoretical straight line denoting proportionality between the quantitative values of this element in substrate and in the tissues. Deviations from the straight line, however, in this as in the succeeding graphs, where the accumulation of an element is considered a function of its concentration in the substrate occur always near the point of maximum growth. Such deviations are probably to be expected, and although at present there is no clear explanation for them other than that suggested, they do not materially detract from the general trends brought out by the analytical data and expressed in graphic form in figures 1 to 6.

Further consideration of figure 1 shows an acceleration of accumulation of magnesium with increase in nitrate concentration in the substrate, and also the highly depressing competitive effect of the mobile potassium ion upon the accumulation of magnesium in the tissues. Both relations are in agreement with Hoagland's hypothesis of ionic penetration. The more sluggish calcium ion appears to have a slightly stimulating effect upon accumulation of magnesium, which also fits into the theory of interionic competition. The phosphate and sulfate ions are unimportant in this effect. As might be expected, the plotted data referring to stem tissue in figure 1, as in the

TABLE 3

Total magnesium, in milligrams per 100 gm. fresh tissue (X_1), allowances for each of the independent ion concentration variables ($X_2' - X_7'$), and the residuals from the statistical analysis (Z')

TREATMENT NUMBER	X_1 MAGNESIUM FOUND		X_2' (Mg^{++})		X_3' (NO_3^-)		X_4' (K^+)		X_5' ($PO_4^{\equiv}$)		X_6' (Ca^{++})		X_7' ($SO_4^{=}$)		Z_7' RESIDUALS	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
1	54.9	37.0	45.7	31.9	1.5	2.6	−0.1	0	0	0	0	0	0	0	7.8	2.5
2	30.9	19.0	45.7	31.9	−11.9	−9.4	−0.1	0	1.5	1.9	0	0	0.4	0.5	−4.7	−5.9
3	69.9	58.0	45.7	31.9	22.6	16.0	−0.1	0	−0.8	−0.9	0	0	0.4	0.5	2.1	10.5
4	26.6	13.7	45.7	31.9	−11.9	−9.4	−0.1	0	−0.8	−0.9	0	0	−0.9	−1.0	−5.4	−6.9
5	69.4	46.4	35.4	26.9	22.6	16.0	7.3	5.8	−0.8	−0.9	7.2	5.8	0.4	0.5	−2.7	−7.7
6	66.4	40.6	35.4	26.9	1.5	2.6	7.3	5.8	0	0	7.2	5.8	0	0	15.0	−0.5
7	32.8	33.4	35.4	26.9	−11.9	−9.4	7.3	5.8	−0.8	−0.9	7.2	5.8	−0.9	−1.0	−3.5	6.2
8	31.8	34.4	35.4	26.9	−11.9	−9.4	7.3	5.8	1.5	1.9	7.2	5.8	0.4	0.5	−8.1	2.9
9	16.8	13.5	35.4	26.9	−11.9	−9.4	−14.4	−11.5	1.5	1.9	−3.6	−2.9	0.4	0.5	9.4	8.0
10	12.0	9.1	35.4	26.9	1.5	2.6	−14.4	−11.5	0	0	−3.6	−2.9	0	0	−6.9	−6.0
11	28.9	19.7	35.4	26.9	22.6	16.0	−14.4	−11.5	−0.8	−0.9	−3.6	−2.9	0.4	0.5	−10.7	−8.4
12	11.9	6.7	35.4	26.9	−11.9	−9.4	−14.4	−11.5	−0.8	−0.9	−3.6	−2.9	−0.9	−1.0	8.1	5.5
13	99.7	59.5	107.5	72.7	−11.9	−9.4	7.3	5.8	−0.8	−0.9	−3.6	−2.9	−0.9	−1.0	1.3	−4.8
14	97.5	82.3	107.5	72.7	1.5	2.6	7.3	5.8	0	0	−3.6	−2.9	0	0	−16.0	4.1
15	145.2	96.5	107.5	72.7	22.6	16.0	7.3	5.8	−0.8	−0.9	−3.6	−2.9	0.4	0.5	11.0	5.3
16	105.3	64.0	107.5	72.7	−11.9	−9.4	7.3	5.8	1.5	1.9	−3.6	−2.9	0.4	0.5	3.3	−4.6

TABLE 4

Soluble magnesium, in milligrams per 100 gm. fresh tissue (X_1), allowances for each of the independent ion concentration variables ($X_2' - X_7'$), and the residuals from the statistical analysis (Z_7')

TREATMENT NUMBER	X_1 MAGNESIUM FOUND		X_2' (Mg^{++})		X_3' (NO_3^-)		X_4' (K^+)		X_5' ($PO_4^{\equiv}$)		X_6' (Ca^{++})		X_7' ($SO_4^{\equiv}$)		Z_7' RESIDUALS	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
1	49.8	20.9	42.6	20.4	2.7	0.4	0.1	0	0	0	0	0	0	0	4.4	0
2	29.3	15.2	42.6	20.4	−10.7	−7.2	0.1	0	1.2	2.8	0	0	0.3	0.7	−4.2	−1.6
3	65.3	35.7	42.6	20.4	18.7	14.0	0.1	0	−0.6	−1.4	0	0	0.3	0.7	4.2	2.0
4	26.2	10.0	42.6	20.4	−10.7	−7.2	0.1	0	−0.6	−1.4	0	0	−0.6	−1.4	−4.6	−0.5
5	54.7	40.9	27.6	22.5	18.7	14.0	4.7	5.0	−0.6	−1.4	4.6	5.0	0.3	0.7	−0.6	−4.9
6	43.7	36.6	27.6	22.5	2.7	0.4	4.7	5.0	0	0	4.6	5.0	0	0	4.1	3.6
7	25.0	26.7	27.6	22.5	−10.7	−7.2	4.7	5.0	−0.6	−1.4	4.6	5.0	−0.6	−1.4	0	4.2
8	24.3	26.0	27.6	22.5	−10.7	−7.2	4.7	5.0	1.2	2.8	4.6	5.0	0.3	0.7	−3.4	−2.8
9	15.7	10.2	27.6	22.5	−10.7	−7.2	−9.4	−10.0	1.2	2.8	−2.3	−2.5	0.3	0.7	9.0	3.9
10	10.0	6.9	27.6	22.5	2.7	0.4	−9.4	−10.0	0	0	−2.3	−2.5	0	0	−8.6	−3.6
11	26.0	16.8	27.6	22.5	18.7	14.0	−9.4	−10.0	−0.6	−1.4	−2.3	−2.5	0.3	0.7	−8.3	−6.6
12	10.7	4.9	27.6	22.5	−10.7	−7.2	−9.4	−10.0	−0.6	−1.4	−2.3	−2.5	−0.6	−1.4	6.7	4.7
13	70.5	38.5	81.6	54.7	−10.7	−7.2	4.7	5.0	−0.6	−1.4	−2.3	−2.5	−0.6	−1.4	−1.6	−8.6
14	87.0	57.5	81.6	54.7	2.7	0.4	4.7	5.0	0	0	−2.3	−2.5	0	0	0.3	−0.4
15	107.0	79.5	81.6	54.7	18.7	14.0	4.7	5.0	−0.6	−1.4	−2.3	−2.5	0.3	0.7	4.5	9.1
16	72.6	54.4	81.6	54.7	−10.7	−7.2	4.7	5.0	1.2	2.8	−2.3	−2.5	0.3	0.7	−2.2	0.9

succeeding figures, show strikingly similar trends, although the orders of magnitude vary considerably.

Figure 2 shows for calcium the same type of direct relation between variations in accumulation in the tissue and ionic concentration in the substrate as was shown in figure 1 for magnesium. Likewise, as was found for the magnesium content of the tissue, increasing calcium content was directly correlated with increasing nitrate ionic concentrations in the substrate. These curves represent the plotted data taken from tables 5 and 6. The slightly accelerating effect of increasing potassium concentration in the substrate upon calcium accumulation in the tissue here appears contradictory to this general

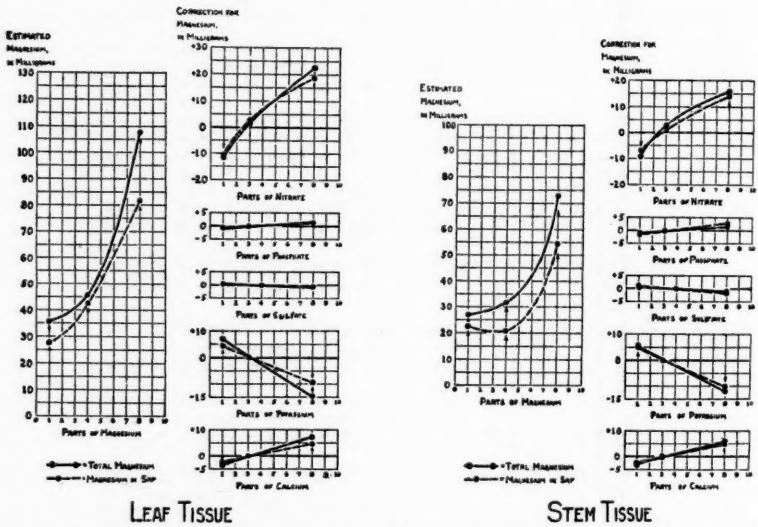

FIG. 1. NET REGRESSION CURVES SHOWING THE EFFECTS OF CONCENTRATION OF THE VARIOUS IONS IN THE SUBSTRATE UPON ACCUMULATION OF MAGNESIUM IN CORN TISSUE
The magnesium found is expressed in milligrams per 100 gm. fresh tissue

hypothesis, but it must be remembered that growth was stimulated by increasing concentrations of both of these ions (1), and that the rate of nitrate penetration is such that the cation competition may be partially eliminated. It is apparent, also, that within the ranges of concentration here considered, the phosphate, sulfate, and magnesium ions respectively had no significant effect upon the accumulation of calcium in the tissues.

With respect to potassium accumulation (fig. 3), the stem tissue analyses show a potassium content per 100 gm. of fresh tissue of approximately the same order of magnitude as that of the leaf tissue. This is probably because the meristematic tissue of corn occurs almost exclusively in the stem aliquot. These curves represent the X' values presented in tables 7 and 8. Increasing

TABLE 5

Soluble calcium, in milligrams per 100 gm. fresh tissue (X_1), allowances for each of the independent ion concentration variables ($X_2' - X_7'$), and the residuals from the statistical analysis (Z_7')

TREATMENT NUMBER	X_1 CALCIUM FOUND		X_2' (Ca^{++})		X_3' (NO_3^-)		X_4' (K^+)		X_5' ($PO_4^{=}$)		X_6' (Mg^{++})		X_7' ($SO_4^{=}$)		Z_7' RESIDUALS	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
1	25.6	10.8	24.6	11.1	−1.3	−2.2	0	0	0	0	0	0	0	0	2.3	1.8
2	24.8	9.5	24.6	11.1	−4.6	−2.1	0	0	1.6	0.7	0	0	0.4	0.2	2.8	−0.5
3	32.1	19.4	24.6	11.1	10.5	6.4	0	0	−0.8	−0.4	0	0	0.4	0.2	−2.6	2.0
4	16.1	4.9	24.6	11.1	−4.6	−2.1	0	0	−0.8	−0.4	0	0	−0.8	−0.4	−2.3	−3.3
5	68.9	46.7	47.8	35.1	10.5	6.4	−0.9	−0.4	−0.8	−0.4	0.4	0.2	0.4	0.2	11.5	5.6
6	42.0	26.6	47.8	35.1	−1.3	−2.2	−0.9	−0.4	0	0	0.4	0.2	0	0	−4.0	−6.1
7	38.3	34.0	47.8	35.1	−4.6	−2.1	−0.9	−0.4	−0.8	−0.4	0.4	0.2	−0.8	−0.4	−2.8	2.0
8	39.9	32.2	47.8	35.1	−4.6	−2.1	−0.9	−0.4	1.6	0.7	0.4	0.2	0.4	0.2	−4.8	−1.5
9	13.9	5.7	13.5	4.8	−4.6	−2.1	1.8	0.8	1.6	0.7	0.4	0.2	0.4	0.2	0.8	1.1
10	16.1	5.5	13.5	4.8	−1.3	−2.2	1.8	0.8	0	0	0.4	0.2	0	0	1.7	1.8
11	20.5	9.2	13.5	4.8	10.5	6.4	1.8	0.8	−0.8	−0.4	0.4	0.2	0.4	0.2	−5.3	−2.8
12	11.4	3.0	13.5	4.8	−4.6	−2.1	1.8	0.8	−0.8	−0.4	0.4	0.2	−0.8	−0.4	1.9	0
13	9.4	2.5	13.5	4.8	−4.6	−2.1	−0.9	−0.4	−0.8	−0.4	−0.9	−0.4	−0.8	−0.4	3.9	1.3
14	10.4	4.4	13.5	4.8	−1.3	−2.2	−0.9	−0.4	0	0	−0.9	−0.4	0	0	0	2.5
15	18.1	5.4	13.5	4.8	10.5	6.4	−0.9	−0.4	−0.8	−0.4	−0.9	−0.4	0.4	0.2	−3.7	−4.9
16	9.5	3.9	13.5	4.8	−4.6	−2.1	−0.9	−0.4	1.6	0.7	−0.9	−0.4	0.4	0.2	0.4	1.0

TABLE 6

Total calcium, in milligrams per 100 gm. fresh tissue (X_1), allowances for each of the independent ion concentration variables ($X_2' - X_7'$), and the residuals from the statistical analysis (Z_7')

TREATMENT NUMBER	X_1 CALCIUM FOUND		X_2' (Ca^{++})		X_3' (NO_3^-)		X_4' (K^+)		X_5' ($PO_4^{=}$)		X_6' (Mg^{++})		X_7' ($SO_4^{=}$)		Z_7' RESIDUALS	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
1	43.6	12.7	43.4	15.2	−2.1	−3.9	0	0	0	0	0	0	0	0	2.3	1.4
2	40.0	17.0	43.4	15.2	−6.0	−2.2	0	0	0.7	1.4	0	0	0.2	0.4	1.7	2.2
3	57.4	24.9	43.4	15.2	14.1	8.4	0	0	−0.4	−0.7	0	0	0.2	0.4	0.1	1.6
4	32.7	6.4	43.4	15.2	−6.0	−2.2	0	0	−0.4	−0.7	0	0	−0.3	−0.8	−4.0	−5.1
5	125.4	60.5	96.5	44.3	14.1	8.4	−1.9	−0.2	−0.4	−0.7	0.9	0.1	0.2	0.4	16.0	8.2
6	87.6	31.3	96.5	44.3	−2.1	−3.9	−1.9	−0.2	0	0	0.9	0.1	0	0	−5.8	−9.0
7	86.8	42.6	96.5	44.3	−6.0	−2.2	−1.9	−0.2	−0.4	−0.7	0.9	0.1	−0.3	−0.8	−2.0	−2.1
8	81.7	43.0	96.5	44.3	−6.0	−2.2	−1.9	−0.2	0.7	1.4	0.9	0.1	0.2	0.4	−8.7	−0.8
9	27.6	6.2	24.5	6.7	−6.0	−2.2	3.8	0.4	0.7	1.4	0.9	0.1	0.2	0.4	3.3	−0.6
10	29.2	6.6	24.5	6.7	−2.1	−3.9	3.8	0.4	0	0	0.9	0.1	0	0	2.1	3.3
11	40.0	12.3	24.5	6.7	14.1	8.4	3.8	0.4	−0.4	−0.7	0.9	0.1	0.2	0.4	−3.1	−3.0
12	20.8	3.3	24.5	6.7	−6.0	−2.2	3.8	0.4	−0.4	−0.7	0.9	0.1	−0.3	−0.8	−1.7	−0.2
13	22.1	6.3	24.5	6.7	−6.0	−2.2	−1.9	−0.2	−0.4	−0.7	−1.8	−0.2	−0.3	−0.8	8.0	3.7
14	20.3	6.7	24.5	6.7	−2.1	−3.9	−1.9	−0.2	0	0	−1.8	−0.2	0	0	1.6	4.3
15	21.7	7.3	24.5	6.7	14.1	8.4	−1.9	−0.2	−0.4	−0.7	−1.8	−0.2	0.2	0.4	−13.0	−7.1
16	19.0	5.1	24.5	6.7	−6.0	−2.2	−1.9	−0.2	0.7	1.4	−1.8	−0.2	0.2	0.4	3.3	−0.8

concentrations of nitrate above the minimum here employed appear to have a somewhat depressing effect upon potassium accumulation, but since the highest yield of the entire series was produced by the solution high in both nitrate and potassium, it is obvious that here the highest growth rates are dealt with, and are accompanied by the most rapid increase in tissue volume. With the relatively mature leaf tissue of treatment 11 (table 1), however, high nitrate concentration gives the highest potassium accumulation, as would be expected in accordance with Hoagland's hypothesis. This particular relation, however, is not indicated by the statistical analysis. High metabolic activity, or rapid growth, would then appear to have a depressing effect upon the quantitative accumulation value of any element concerned and would tend to minimize the effect of rapid penetration of ions upon their accumula-

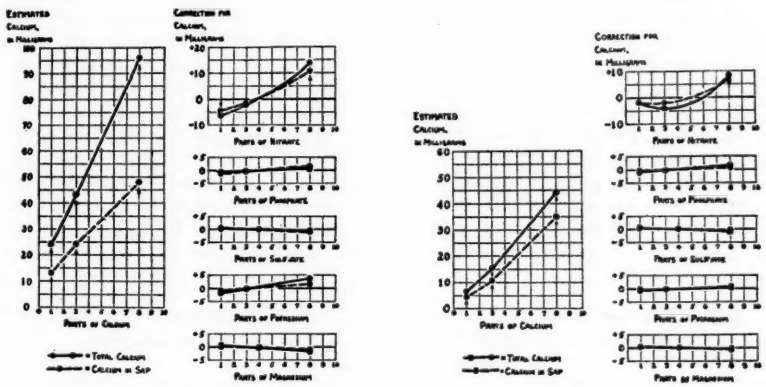

FIG. 2. NET REGRESSION CURVES SHOWING THE EFFECTS OF CONCENTRATION OF THE VARIOUS IONS IN THE SUBSTRATE UPON ACCUMULATION OF CALCIUM IN CORN TISSUE
The calcium found is expressed in milligrams per 100 gm. fresh tissue

tion. Contrastingly, a low growth rate would appear to intensify the effect of the rate of penetration upon accumulation.

Likewise there is a tendency for increasing calcium concentration in the substrate to depress correspondingly the accumulation of potassium in the stem tissue. The phosphate and sulfate ions within the limits of concentration here employed are without significant effect upon the accumulation of potassium, but increasing concentrations of magnesium have a very slightly accelerating effect in stem tissues only.

The data for the potassium analyses show that not all of the potassium of these plants was found in the expressed sap. Considerable quantities of potassium, particularly with respect to high concentrations, were found to be in some manner bound, a finding contrary to that of Morris and Sayre

TABLE 7

Soluble potassium, in milligrams per 100 gm. fresh tissue (X_1), allowances for each of the independent ion concentration variables ($X_2' - X_7'$), and the residuals from the statistical analysis (Z_7')

TREATMENT NUMBER	X_1 POTASSIUM FOUND		X_2' (K^+)		X_3' (NO_3^-)		X_4' (Ca^{++})		X_5' ($PO_4^{\equiv}$)		X_6' (Mg^{++})		X_7' ($SO_4^{\equiv}$)		Z_7' RESIDUALS	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
1	260	162	314	204	−19	−23	0	1	0	0	0	0	0	0	−35	−20
2	327	192	314	204	13	23	0	1	−1	5	0	0	0	1	1	−42
3	243	176	314	204	−5	−25	0	1	0	−3	0	0	0	1	−66	−2
4	428	287	314	204	13	23	0	1	0	−3	0	0	0	−2	101	64
5	148	77	185	137	−5	−25	−4	−22	0	−3	−1	−6	0	1	−27	−5
6	140	51	185	137	−19	−23	−4	−22	0	0	−1	−6	0	0	−21	−35
7	180	135	185	137	13	23	−4	−22	0	−3	−1	−6	0	−2	−13	8
8	259	169	185	137	13	23	−4	−22	−1	5	−1	−6	0	1	67	31
9	363	414	389	340	13	23	2	11	−1	5	−1	−6	0	1	−39	40
10	380	390	389	340	−19	−23	2	11	0	0	−1	−6	0	0	9	68
11	467	320	389	340	−5	−25	2	11	0	−3	−1	−6	0	1	82	2
12	346	259	389	340	13	23	2	11	0	−3	−1	−6	0	−2	−57	−104
13	174	212	185	137	13	23	2	11	0	−3	2	13	0	−2	−28	33
14	218	124	185	137	−19	−23	2	11	0	0	2	13	0	0	48	−14
15	195	145	185	137	−5	−25	2	11	0	−3	2	13	0	1	11	11
16	170	156	185	137	13	23	2	11	−1	5	2	13	0	1	−31	−34

TABLE 8

Total potassium, in milligrams per 100 gm. fresh tissue (X_1), allowances for each of the independent ion concentration variables ($X_2' - X_7'$), and the residuals from the statistical analysis (Z_7')

TREATMENT NUMBER	X_1 POTASSIUM FOUND		X_2' (K^+)		X_3' (NO_3^-)		X_4' (Ca^{++})		X_5' ($PO_4^{\equiv}$)		X_6' (Mg^{++})		X_7' ($SO_4^{\equiv}$)		Z_7' RESIDUALS	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
1	337	255	360	319	−16	−22	0	0	0	0	0	0	0	0	−7	−42
2	384	328	360	319	20	53	0	0	0	−13	0	0	0	−4	4	−27
3	269	187	360	319	−24	−84	0	0	0	7	0	0	0	−4	−67	−51
4	450	507	360	319	20	53	0	0	0	7	0	0	0	7	70	121
5	205	78	248	169	−24	−84	−13	−41	0	7	−4	−11	0	−4	−2	42
6	207	52	248	169	−16	−22	−13	−41	0	0	−4	−11	0	0	−8	−43
7	224	141	248	169	20	53	−13	−41	0	7	−4	−11	0	7	−27	−43
8	294	192	248	169	20	53	−13	−41	0	−13	−4	−11	0	−4	43	39
9	452	482	454	461	20	53	7	20	0	−13	−4	−11	0	−4	−25	−24
10	460	548	454	461	−16	−22	7	20	0	0	−4	−11	0	0	19	100
11	492	380	454	461	−24	−84	7	20	0	7	−4	−11	0	−4	59	−9
12	423	477	454	461	20	53	7	20	0	7	−4	−11	0	7	−54	−60
13	293	256	248	169	20	53	7	20	0	7	7	21	0	7	11	−21
14	240	174	248	169	−16	−22	7	20	0	0	7	21	0	0	−6	−14
15	245	151	248	169	−24	−84	7	20	0	7	7	21	0	−4	7	22
16	261	258	248	169	20	53	7	20	0	−13	7	21	0	−4	−21	12

(11). The data support the findings of McGeorge (10), however, that the adsorptive capacity of the colloidal plant complex can be at least partially saturated with potassium ions. Morris and Sayre have based their conclusions upon analytical data from soil-grown corn plants in which the ratio of potassium to the other bases was not given. It is evident that this ratio was not favorable for partial saturation of the colloidal plant complex by potassium. In sand culture work, this ratio may be under much better control than in soil, and the sodium ion may be approximately eliminated from the culture solution.

Consideration of figure 4 further substantiates the conclusion that within the limits of concentrations here used metabolically active ions may have

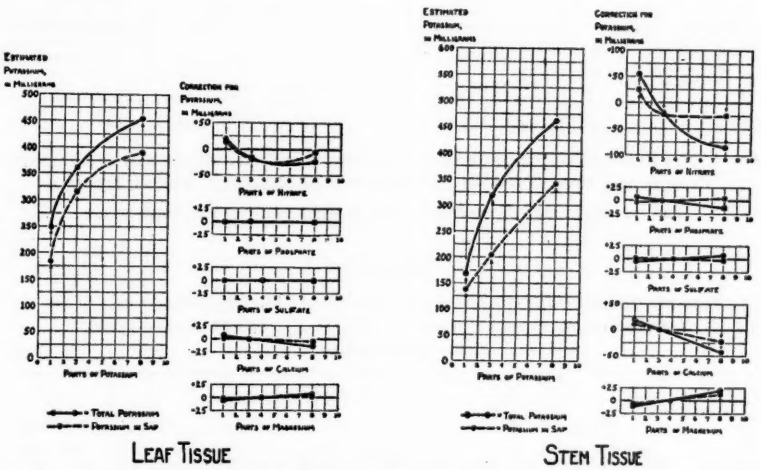

FIG. 3. NET REGRESSION CURVES SHOWING THE EFFECTS OF CONCENTRATION OF THE VARIOUS IONS IN THE SUBSTRATE UPON ACCUMULATION OF POTASSIUM IN CORN TISSUE

The potassium found is expressed in milligrams per 100 gm. fresh tissue

an apparent depressing effect upon accumulation of other metabolically active elements, both total and soluble. This is indicated by the fact that both potassium and calcium ions, which with increasing concentrations were responsible for an increased growth rate in this series of plants (1), have an apparently depressing effect upon the accumulation of nitrogen. The other ions are without demonstrable effect in this respect. The graphs in figure 4 are plotted from the X' values presented in tables 9 and 10.

Accumulation of phosphorus (fig. 5, plotted from data presented in tables 11 and 12) was depressed slightly with increasing concentrations of calcium in the substrate but was relatively unaffected by change in the concentration of the other ions. The curve representing the allowance for potassium shows the characteristic dip associated with high growth rates of these plants in

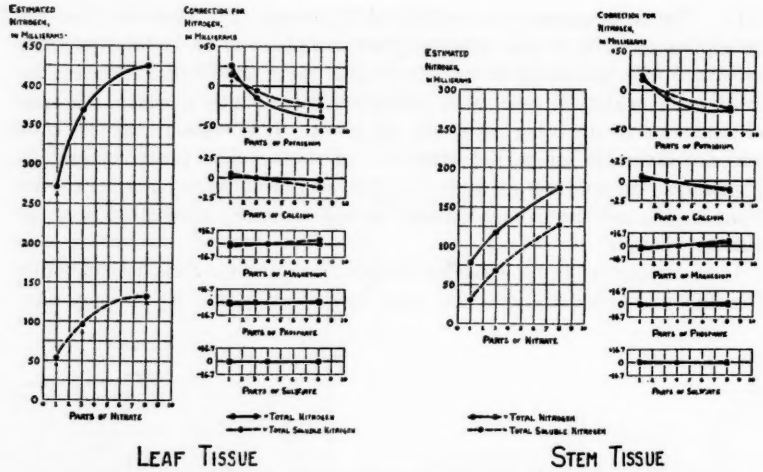

FIG. 4. NET REGRESSION CURVES SHOWING THE EFFECTS OF CONCENTRATION OF THE VARIOUS IONS IN THE SUBSTRATE UPON ACCUMULATION OF NITROGEN IN CORN TISSUE

The nitrogen found is expressed in milligrams per 100 gm. fresh tissue

TABLE 9

Total nitrogen, in milligrams per 100 gm. fresh tissue (X_1), allowances for each of the independent ion concentration variables ($X_2' - X_7'$), and the residuals from the statistical analysis (Z_7')

TREATMENT NUMBER	X_1 NITROGEN FOUND		X_2' (NO_3^-)		X_3' (K^+)		X_4' ($PO_4^{\equiv}$)		X_5' (Ca^{++})		X_6' ($SO_4^{\equiv}$)		X_7' (Mg^{++})		Z_7' RESIDUALS	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
1	324	91.8	368	117.0	−14	−11.3	0	0	0	0	0	0	0	0	−30	−13.9
2	261	72.3	271	78.5	−14	−11.3	1	1.0	0	0	0	0.2	0	0	3	3.9
3	441	171.8	425	173.0	−14	−11.3	−1	−0.5	0	0	0	0.2	0	0	31	10.4
4	252	65.6	271	78.5	−14	−11.3	−1	−0.5	0	0	0	−0.5	0	0	−4	−0.6
5	470	177.0	425	173.0	26	18.2	−1	−0.5	−2	−11.6	0	0.2	0	−2.9	22	0.6
6	399	114.2	368	117.0	26	18.2	0	0	−2	−11.6	0	0	0	−2.9	7	−6.5
7	276	77.6	271	78.5	26	18.2	−1	−0.5	−2	−11.6	0	−0.5	0	−2.9	−18	−3.6
8	286	92.8	271	78.5	26	18.2	1	1.0	−2	−11.6	0	0.2	0	−2.9	−10	9.4
9	244	68.0	271	78.5	−37	−25.3	1	1.0	1	5.9	0	0.2	0	−2.9	8	10.6
10	332	78.1	368	117.0	−37	−25.3	0	0	1	5.9	0	0	0	−2.9	0	−16.6
11	354	141.0	425	173.0	−37	−25.3	−1	−0.5	1	5.9	0	0.2	0	−2.9	−34	−9.4
12	257	70.2	271	78.5	−37	−25.3	−1	−0.5	1	5.9	0	−0.5	0	−2.9	23	15.0
13	291	96.8	271	78.5	26	18.2	−1	−0.5	1	5.9	0	−0.5	0	5.8	−6	−10.6
14	418	183.8	368	117.0	26	18.2	0	0	1	5.9	0	0	0	5.8	23	36.9
15	435	201.0	425	173.0	26	18.2	−1	−0.5	1	5.9	0	0.2	0	5.8	−16	−1.6
16	300	85.3	271	78.5	26	18.2	1	1.0	1	5.9	0	0.2	0	5.8	1	−24.3

TABLE 10

Soluble nitrogen, in milligrams per 100 gm. fresh tissue (X_1), allowances for each of the independent ion concentration variables ($X'_2 - X'_7$), and the residuals from the statistical analysis (Z'_7)

TREATMENT NUMBER	X_1 NITROGEN FOUND		X'_2 (NO_3^-)		X'_3 (K^+)		X'_4 ($PO_4^{=}$)		X'_5 (Ca^{++})		X'_6 ($SO_4^{=}$)		X'_7 (Mg^{++})		Z'_7 RESIDUALS	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
1	75.3	50.4	96.0	67.2	−5.9	−4.0	0	0	0	0	0	0	0	0	−14.8	−12.8
2	57.3	27.8	54.0	30.7	−5.9	−4.0	0	−0.6	0	0	0	−0.1	0	0	9.2	1.8
3	135.6	132.0	132.5	126.9	−5.9	−4.0	0	0.3	0	0	0	−0.1	0	0	9.0	8.9
4	44.8	29.4	54.0	30.7	−5.9	−4.0	0	0.3	0	0	0	0.3	0	0	−3.3	2.1
5	142.2	126.0	132.5	126.9	14.7	13.2	0	0.3	−11.0	−12.9	0	−0.1	−2.7	−3.2	8.7	2.0
6	88.9	58.0	96.0	67.2	14.7	13.2	0	0	−11.0	−12.9	0	0	−2.7	−3.2	−8.1	−6.1
7	57.7	30.0	54.0	30.7	14.7	13.2	0	0.3	−11.0	−12.9	0	0.3	−2.7	−3.2	2.7	1.8
8	51.6	29.0	54.0	30.7	14.7	13.2	0	−0.6	−11.0	−12.9	0	−0.1	−2.7	−3.2	−3.4	2.1
9	45.5	25.2	54.0	30.7	−23.6	−22.3	0	−0.6	5.5	6.5	0	−0.1	−2.7	−3.2	12.3	13.8
10	64.5	37.6	96.0	67.2	−23.6	−22.3	0	0	5.5	6.5	0	0	−2.7	−3.2	−10.7	−11.0
11	92.3	91.6	132.5	126.9	−23.6	−22.3	0	0.3	5.5	6.5	0	−0.1	−2.7	−3.2	−0.2	−16.9
12	50.7	26.4	54.0	30.7	−23.6	−22.3	0	0.3	5.5	6.5	0	0.3	−2.7	−3.2	17.5	13.0
13	62.9	39.5	54.0	30.7	14.7	13.2	0	0.3	5.5	6.5	0	0.3	5.6	6.3	−16.9	−17.6
14	155.5	123.0	96.0	67.2	14.7	13.2	0	0	5.5	6.5	0	0	5.6	6.3	33.7	30.0
15	160.0	158.2	132.5	126.9	14.7	13.2	0	0.3	5.5	6.5	0	−0.1	5.6	6.3	1.7	5.3
16	61.6	38.6	54.0	30.7	14.7	13.2	0	−0.6	5.5	6.5	0	−0.1	5.6	6.3	−18.2	−17.2

a substrate having a favorable cation balance. Phorphorus accumulates in the leaf tissue largely without relation to nitrate ion concentration in the substrate.

In the stem tissue an apparent inverse relationship exists between the phosphorus content of the tissue and the nitrate ion concentration of the substrate. This relationship is explainable according to the competitive anion hypothesis, because, as will be shown in a subsequent paper, this tissue contained a considerable amount of nitrate nitrogen when grown in solutions high in nitrate. In the leaf tissue the nitrate nitrogen is rapidly reduced in the process of nitrogen assimilation, but in the stem tissue a sufficient excess of unreduced nitrate ions may be present to depress the accumulation of

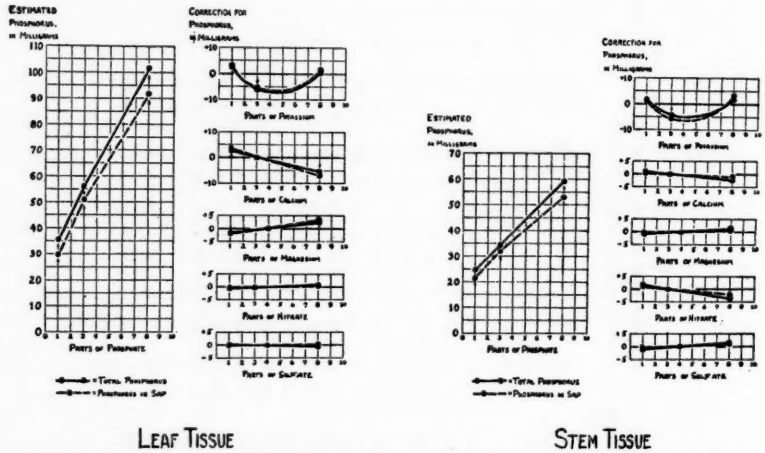

FIG. 5. NET REGRESSION CURVES SHOWING THE EFFECTS OF CONCENTRATION OF THE VARIOUS IONS IN THE SUBSTRATE UPON ACCUMULATION OF PHOSPHORUS IN CORN TISSUE

The phosphorus found is expressed in milligrams per 100 gm. fresh tissue

phosphorus, and even here the depressing effect of the nitrate is relatively slight as indicated in figure 5.

Accumulation of sulfur within the tissues, as shown in figure 6, which presents graphically the X' values given in table 13, was apparently depressed by increasing concentrations of nitrate and calcium in the substrate and slightly increased with increasing concentrations of potassium and phosphate.

The relations between the sulfur content of the tissue and potassium and nitrate concentrations in the substrate, directly support Hoagland's hypothesis. The relations between the sulfur content and the calcium and phosphate nutrient concentrations, however, are not explainable from this point of view. It is quite possible that the calcium salts of low solubility, e.g., $CaSO_4$, may limit the quantity of calcium which may pass from the roots into the stem

TABLE 11

Total phosphorus, in milligrams per 100 gm. fresh tissue (X_1), allowances for each of the independent ion concentration variables ($X_2' - X_7'$), and the residuals from the statistical analysis (Z_i')

TREATMENT NUMBER	X_1 PHOSPHORUS FOUND		X_2' ($PO_4^{=}$)		X_3' (K^+)		X_4' (NO_3^-)		X_5' (Ca^{++})		X_6' ($SO_4^{=}$)		X_7' (Mg^{++})		Z_i' RESIDUALS	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
1	41.0	31.4	56.3	34.3	−6.1	−4.2	0	0	0	0	0	0	0	0	−9.2	1.3
2	91.7	48.0	101.5	59.1	−6.1	−4.2	−0.3	1.7	0	0	0.2	−0.8	0	0	−3.6	−7.8
3	36.3	18.7	35.6	24.8	−6.1	−4.2	0.6	−3.4	0	0	0.2	−0.8	0	0	6.0	2.3
4	35.5	28.1	35.6	24.8	−6.1	−4.2	−0.3	1.7	0	0	−0.4	1.6	0	0	6.7	4.2
5	33.0	15.8	35.6	24.8	2.9	1.4	0.6	−3.4	−5.2	−2.3	0.2	−0.8	−1.3	−0.6	0.2	−3.3
6	48.3	31.1	54.3	34.3	2.9	1.4	0	0	−5.2	−2.3	0	0	−1.3	−0.6	−4.4	−1.7
7	32.4	27.3	35.6	24.8	2.9	1.4	−0.3	1.7	−5.2	−2.3	−0.4	1.6	−1.3	−0.6	1.1	0.6
8	100.2	62.6	101.5	59.1	2.9	1.4	−0.3	1.7	−5.2	−2.3	0.2	−0.8	−1.3	−0.6	2.4	4.1
9	106.7	65.3	101.5	59.1	0.5	1.5	−0.3	1.7	2.6	1.2	0.2	−0.8	−1.3	−0.6	3.5	3.2
10	59.1	37.3	56.3	34.3	0.5	1.5	0	0	2.6	1.2	0	0	−1.3	−0.6	1.0	0.9
11	34.9	21.9	35.6	24.8	0.5	1.5	0.6	−3.4	2.6	1.2	0.2	−0.8	−1.3	−0.6	−3.3	−0.8
12	36.3	26.9	35.6	24.8	0.5	1.5	−0.3	1.7	2.6	1.2	−0.4	1.6	−1.3	−0.6	−0.4	−3.3
13	36.1	30.0	35.6	24.8	2.9	1.4	−0.3	1.7	2.6	1.2	−0.4	1.6	2.6	1.1	−6.9	−1.8
14	76.9	37.4	56.3	34.3	2.9	1.4	0	0	2.6	1.2	0	0	2.6	1.1	12.5	−0.6
15	41.3	25.8	35.6	24.8	2.9	1.4	0.6	−3.4	2.6	1.2	0.2	−0.8	2.6	1.1	−3.2	1.5
16	107.2	64.6	101.5	59.1	2.9	1.4	−0.3	1.7	2.6	1.2	0.2	−0.8	2.6	1.1	−2.3	0.9

TABLE 12

Soluble phosphorus, in milligrams per 100 gm. fresh tissue (X_1), allowances for each of the independent ion concentration variables ($X_2' - X_7'$), and the residuals from the statistical analysis (Z_7')

TREATMENT NUMBER	X_1 PHOSPHORUS FOUND		X_2' ($PO_4^{=}$)		X_3' (K^+)		X_4' (NO_3^-)		X_5' (Ca^{++})		X_6' ($SO_4^{=}$)		X_7' (Mg^{++})		Z_7' RESIDUALS	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
1	35.0	30.7	51.2	32.0	−5.6	−5.7	0	0	0	0	0	0	0	0	10.6	4.4
2	82.5	34.3	91.8	52.8	−5.6	−5.7	0.2	1.2	0	0	−0.1	−0.6	0	0	−3.8	−13.4
3	29.2	16.2	30.0	21.3	−5.6	−5.7	−0.3	−2.2	0	0	−0.1	−0.6	0	0	5.2	3.4
4	33.9	23.4	30.0	21.3	−5.6	−5.7	0.2	1.2	0	0	0.2	1.2	0	0	9.1	5.4
5	25.7	14.2	30.0	21.3	2.1	1.2	−0.3	−2.2	−6.6	−1.1	−0.1	−0.6	−1.6	−0.3	2.2	−4.1
6	41.1	30.5	51.2	32.0	2.1	1.2	0	0	−6.6	−1.1	0	0	−1.6	−0.3	−4.0	−1.3
7	22.4	24.1	30.0	21.3	2.1	1.2	0.2	1.2	−6.6	−1.1	0.2	1.2	−1.6	−0.3	−1.9	0.6
8	89.2	58.5	91.8	52.8	2.1	1.2	0.2	1.2	−6.6	−1.1	−0.1	−0.6	−1.6	−0.3	3.4	5.3
9	101.8	60.8	91.8	52.8	1.3	3.4	0.2	1.2	3.3	0.5	−0.1	−0.6	−1.6	−0.3	6.9	3.8
10	53.8	36.0	51.2	32.0	1.3	3.4	0	0	3.3	0.5	0	0	−1.6	−0.3	−0.4	0.4
11	28.6	20.1	30.0	21.3	1.3	3.4	−0.3	−2.2	3.3	0.5	−0.1	−0.6	−1.6	−0.3	−4.0	−2.0
12	31.0	25.1	30.0	21.3	1.3	3.4	0.2	1.2	3.3	0.5	0.2	1.2	−1.6	−0.3	−2.4	−2.2
13	34.3	21.6	30.0	21.3	2.1	1.2	0.2	1.2	3.3	0.5	0.2	1.2	3.3	0.5	−4.8	−4.3
14	74.8	30.7	51.2	32.0	2.1	1.2	0	0	3.3	0.5	0	0	3.3	0.5	14.9	−3.5
15	35.3	23.5	30.0	21.3	2.1	1.2	−0.3	−2.2	3.3	0.5	−0.1	−0.6	3.3	0.5	−3.0	2.8
16	93.7	60.2	91.8	52.8	2.1	1.2	0.2	1.2	3.3	0.5	−0.1	−0.6	3.3	0.5	−6.9	4.6

TABLE 13

Soluble sulfur, in milligrams per 100 gm. fresh tissue (X_1), allowances for each of the independent ion concentration variables ($X_2' - X_7'$), and the residuals from the statistical analysis (Z_7')

TREATMENT NUMBER	X_1 SULFUR FOUND		X_2' ($SO_4^{=}$)		X_3' (K^+)		X_4' (NO_3^-)		X_5' (Ca^{++})		X_6' ($PO_4^{=}$)		X_7' (Mg^{++})		Z_7' RESIDUALS	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
1	39.8	12.9	30.2	10.6	2.0	−0.7	0	0	0	0	0	0	0	0	7.6	3.0
2	25.8	8.2	20.9	8.4	2.0	−0.7	2.2	1.2	0	0	2.4	1.3	0	0	−1.7	−2.0
3	15.3	5.4	20.9	8.4	2.0	−0.7	−4.7	−2.3	0	0	−1.2	−0.7	0	0	−1.7	0.7
4	53.6	14.7	54.5	16.6	2.0	−0.7	2.2	1.2	0	0	−1.2	−0.7	0	0	−3.9	−1.7
5	13.1	5.1	20.9	8.4	−2.4	−0.8	−4.7	−2.3	−3.8	−0.6	−1.2	−0.7	−1.1	−0.1	5.4	1.2
6	23.2	9.0	30.2	10.6	−2.4	−0.8	0	0	−3.8	−0.6	0	0	−1.1	−0.1	0.3	−0.1
7	38.0	14.5	54.5	16.6	−2.4	−0.8	2.2	1.2	−3.8	−0.6	−1.2	−0.7	−1.1	−0.1	−10.2	−1.1
8	22.8	9.8	20.9	8.4	−2.4	−0.8	2.2	1.2	−3.8	−0.6	2.4	1.3	−1.1	−0.1	4.6	0.4
9	28.8	13.9	20.9	8.4	3.0	2.4	2.2	1.2	2.0	0.3	2.4	1.3	−1.1	−0.1	−0.6	0.4
10	41.0	15.1	30.2	10.6	3.0	2.4	0	0	2.0	0.3	0	0	−1.1	−0.1	6.9	1.9
11	17.2	5.8	20.9	8.4	3.0	2.4	−4.7	−2.3	2.0	0.3	−1.2	−0.7	−1.1	−0.1	−1.7	−2.2
12	55.8	18.9	54.5	16.6	3.0	2.4	2.2	1.2	2.0	0.3	−1.2	−0.7	−1.1	−0.1	−3.6	−0.8
13	75.7	20.8	54.5	16.6	−2.4	−0.8	2.2	1.2	2.0	0.3	−1.2	−0.7	2.1	0.2	18.5	4.0
14	17.0	5.4	30.2	10.6	−2.4	−0.8	0	0	2.0	0.3	0	0	2.1	0.2	−14.9	−4.9
15	14.3	5.6	20.9	8.4	−2.4	−0.8	−4.7	−2.3	2.0	0.3	−1.2	−0.7	2.1	0.2	−2.4	0.5
16	25.0	11.1	20.9	8.4	−2.4	−0.8	2.2	1.2	2.0	0.3	2.4	1.3	2.1	0.2	−2.2	0.5

and leaf tissues, but, aside from theoretical grounds, there is no direct evidence in the present work to support such an explanation.

The interrelationships between element content of the tissues and nutrient ion concentrations which have been indicated as a result of the statistical treatment of the analytical data presented here are in many cases very definite and are in good agreement with the experimental results and hypothetical considerations of other workers. Those relationships which are apparently at variance with the general hypothesis of interionic relations previously mentioned have been discussed. It is to be emphasized that the conclusions which have been suggested should be regarded as tentative and should be taken as established only for the particular sets of experimental conditions

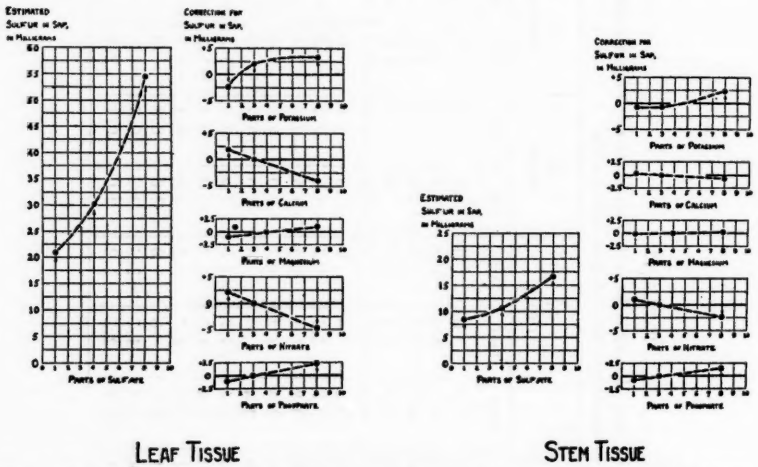

FIG. 6. NET REGRESSION CURVES SHOWING THE EFFECTS OF CONCENTRATION OF THE VARIOUS IONS IN THE SUBSTRATE UPON ACCUMULATION OF SULFUR IN CORN TISSUE
The sulfur found is expressed in milligrams per 100 gm. fresh tissue

herein described. Furthermore it should be expected that other species under the same experimental setup, or the same species in a markedly different environment, might yield results somewhat at variance with those here set down. Such a prediction, however, can be verified only by further investigation.

SUMMARY

Corn plants were grown in sand cultures in which were used a series of nutrient solutions with different proportions of the essential ions, potassium, calcium, magnesium, phosphate, nitrate, and sulfate. Leaf and stem tissues from each treatment were harvested separately, and each fraction was analyzed for soluble and total content of potassium, calcium, magnesium, phosphorus,

nitrogen, and sulfur. The data were analyzed statistically in order to determine the relation between the concentration of the nutrient ions in the substrate and their rates of penetration and accumulation in the tissues of corn plants.

It was found that the most important single condition concerned with the penetration and accumulation in the plant tissues of each of the elements investigated, is its absolute concentration in the nutrient solution. It was further found that the content of any element in the tissues may be directly or inversely related to the concentration of other elements in the substrate, depending upon certain of their known chemical properties and the nature and degree of their physiological importance. Many of the interrelations between the accumulation of elements in the tissues and ionic substrate concentrations support the hypothesis suggested by Hoagland et al. of ionic penetration, whereas others apparently fail to do so.

The general trends of these relations in stem tissues and leaf tissues are very similar, although the order of magnitude of values differs considerably. Some of the more important specific relations indicated by means of the statistical treatment of data are briefly summarized.

Within the range of ion concentrations in the culture solution here employed:

Magnesium content of corn tissues was directly related to variations in the nitrate and calcium ion concentrations in the substrate, high magnesium content corresponding to high nitrate and high calcium ion concentrations, and low magnesium content corresponding to low concentrations of these ions; magnesium content of the tissues was affected in the opposite way by variations in the potassium ion concentrations. Magnesium contents of the tissues were unaffected by variations in the phosphate and sulfate ion concentrations.

Calcium content of corn tissues was directly affected by variations in the nitrate ion concentrations in the substrate, high calcium content being associated with high nitrate ion concentration and low calcium content with low nitrate ion concentrations, but was unaffected by variations in the concentrations of other ions in the substrate.

Potassium content of corn tissues was inversely related to variations in nitrate and calcium ion concentrations in the substrate, high potassium content corresponding to low concentrations of these ions and *vice versa*, but appeared to be comparatively unrelated to variations in the substrate concentrations of other ions.

Nitrogen content of corn tissues was inversely affected by variations in concentrations of potassium and calcium ions in the substrate, high nitrogen content being associated with low concentrations of these ions and *vice versa*. Nitrogen content of tissues was unaffected by variations in the concentration of other ions.

Phosphorus content of corn tissues was relatively unaffected by variations in the concentration of any of the nutrient ions in the substrate except by those of the phosphate ion itself.

Sulfur content of corn tissues was inversely related to variations in the concentrations of calcium and nitrate ions in the substrate, high sulfur content corresponding to low concentrations of calcium and nitrate ions in the substrate, and low sulfur content corresponding to high calcium and nitrate ion concentrations; the sulfur content was relatively unaffected by variations in the concentrations of other ions.

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FACTORS INFLUENCING THE RATE OF DECOMPOSITION OF DIFFERENT TYPES OF PLANT TISSUE IN SOIL, AND THE EFFECT OF THE PRODUCTS ON PLANT GROWTH[1]

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This study is devoted particularly to decomposition of decaying plants which yield the fertilizing constituents so necessary to the proper growth of other plants. It will become evident that the rate of decomposition of a plant is governed by the ratio of carbohydrates to nitrogenous compounds and that this ratio depends, in turn, upon the typical chemical make-up of the species and upon the stage of growth of a plant at the time it is cut and left to decay.[2]

Generally speaking, the older the plant, the larger is the proportion of carbohydrates in its composition; and types of carbohydrates are more resistant to decomposition by fungi, bacteria, and enzymes than are nitrogenous compounds. Furthermore, a high ratio of carbohydrates to nitrogenous compounds delays the time at which the latter—the fertilizing constituents of the decaying plant—are available for the sustenance of other plants, for as long as the bacteria and fungi of decay find carbohydrates to feed upon, they will come between the growing plant and the nitrogenous compounds in order to consume these latter in themselves.

OBJECT

The object of the investigation was to establish, by experimental methods, principles by which the characteristics and relative values of natural fertilizers (humus) could be estimated in advance of use from their known or discoverable chemical constituents. Such principles would help the agriculturist to maintain the fertility and increase the yield of his land by proper use of his own resources rather than by recourse to commercial fertilizers. With this principal object are combined certain incidental objects growing logically out of the results of the investigation.

[1] Contribution No. 308 of the Massachusetts Agricultural Experiment Station.

[2] The proportions of pentosan and nitrogenous compounds in various plants might be made the basis of a system of plant classification. Although no clear increase of nitrogenous content occurs in the evolutionary series—fungi contain little nitrogen, for example; ferns show somewhat larger proportions; and the dicotyledons have still more; but the monocotyledons show a diminution of nitrogenous content—yet the proportion of pentosans rises steadily throughout the series.

In more detail these objects may be stated as follows:

To determine in two distinct ways, the rates of decomposition, in both soil and sand cultures, of various plants widely used by agriculturists:

By measuring in the laboratory the amount of ammonia and nitrates liberated in the soil or sand culture at given intervals during the period of rotting by the various plants;

By estimating, from effects upon the growth of barley in the greenhouse, the rate at which ammonia and nitrates are successively liberated by the various plants in the soil or sand culture.

In both these methods the total content of carbon, nitrogen, pentosans, and lignin of each plant at a certain stage of growth was first determined. The following factors were kept constant for all samples and tests: the significant chemical constitution of soil and sand cultures; and the amount of nitrogen in each sample of plant tissue left to decay (whatever the total bulk of the sample). The first constant was the result of determinations of the amounts of nitrogen and carbon in the soil cultures (both elements were excluded from the sand cultures); the second constant was the result of determinations of the percentage of nitrogen normally present in each of the samples which were to be rotted in the cultures.

To compare the relative proportions of certain constituents (especially pentosans) of the plants with their relative positions in the classifications of Bessey and Bessey (2), Clements and Showalter (3) and Conard (4), who arrange plants in an evolutionary series determined by morphological rather than chemical considerations; that is, to show a correlation between classification of plants in accordance with their relative contents of pentosans or nitrogen and classifications in accordance with the evolution of their forms.

To determine the relation of the nitrogenous content of a plant to the proportion of silica and lime in the tissue thereof.

To show that correlations (either positive or negative) exist among all the characteristics of plants studied in this experiment.

Rates of decomposition (especially liberation of ammonia and nitrates);

Rates of beneficial effects of these plants when allowed to rot, upon other living plants;

Proportions of nitrogenous compounds to carbohydrates (especially pentosans and lignin) in the original make-up of plants;

Proportions of silica and lime in the tissues of plants;

Classification of plants in evolutionary series.

EXPERIMENTAL

The following plant materials, listed in the order in which the investigation was made, were tested:

1. Sudan grass—*Sorghum vulgare sudanense*
2. German millet—*Setaria italica* L.
3. Oat straw—*Avena sativa* L.
4. Rye straw—*Secale cereale* L.
5. Timothy—*Phleum pratense* L.
6. Corn stover—*Zea mays* L.
7. Red clover—*Trifolium pratense* L.
8. Alfalfa—*Medicago sativa* L.
9. Buckwheat hulls—*Fagopyrum esculentum* Moench.
10. Cottonseed hulls—*Gossypium* var. L.
11. *Andropogon sorghum*, var. L.
12. Tobacco (stems and leaves)—*Nicotiana tabacum* L

13. Fungi—*Polyporus betulinus* Micheli.
14. Seaweed—*Ascophyllum nodosum*
15. Club moss—*Lycopodium complanatum* L.
16. Horsetail—*Equisetum arvense* L.
17. Fern—*Osmundae cinnamonea* L.
18. Buttercup—*Ranunculus acris* L.
19. Buckwheat—*Fagopyrum esculentum* Moench.
20. Knotweed—*Polygonum aviculare* L.
21. Smartweed—*Polygonum persicaria* L.
22. Pepper—*Capsicum annuum*
23. Potato top—*Solanum tuberosum* L.
24. Tomato top—*Lycopersicon esculentum* Mill.
25. Jerusalem artichoke—*Helianthus tuberosus* L.
26. Bidens—*Bidens frondosa* L.
27. Ragweed—*Ambrosia artemesifolia* L.
28. Xanthium—*Xanthium canadense* Mill.
29. Pursley—*Portulaca oleracea* L.
30. Amaranth—*Amaranthus retroflexus* L.
31. Cabbage—*Brassica oleracea* L.
32. Onion—*Ailium cepa* L.
33. Orchard grass—*Dactylis glomerata* L.
34. Lamb's-quarters—*Chenopodium album* L.
35. Kentucky bluegrass—*Poa pratensis* L.
36. Turnip top—*Brassica rapa* L.
37. Canada bluegrass—*Poa compressa* L.
38. Sweet clover—*Melilotus alba* D.
39. Sedge—*Carex lupulinus* Muhl.
40. Sedge—*Juncus effusus* L.
41. Haircap moss—*Polytrichum commune* L.
42. Sedge—*Carex stricta* Lam.
43. Witch grass—*Agropyron repens* L. (Beauv).
44. *Gladiolus* var.
45. Redtop—*Agrostis alba* L.

The plant parts used were finely ground and incorporated into sand and into soil in 1-gallon stone jars. The experiment was carried on over a period of 8 weeks and in a few instances 9 weeks. During this period an analysis was made to determine the rate of decomposition as indicated by the amount of ammonia and nitrate nitrogen formed.

The soil used was Merrimac sandy loam from the experiment station farm. It was allowed to dry considerably so as to be easily screened and pulverized. Glass sand was obtained from the Berkshire Sand Company and treated similarly to the soil. In the lime treatments the equivalent of 2 tons per acre was used.

A sufficient amount of soil was weighed to provide 8 pounds for each pot used in the series. To it was added 4.5 gm. of moncalcium phosphate and 1.5 gm. of potassium sulfate, which were constant throughout the investigation. After a thorough mixing 16 pounds was weighed, and into it was put the ground plant tissue. The mixture was then divided equally into two pots.

Each pot contained 8 pounds of slightly moist soil having a total nitrogen

content of 0.143 per cent, or 5.19 gm. and a carbon content of 1.63 per cent, or 59.15 gm. The total nitrogen content of the plant tissue was determined, and equivalent quantities of nitrogen were added from each source to make 0.41 gm. It was unfortunate that in this investigation equivalent quantities of carbon, lignin, cellulose, etc. could not be added and at the same time have an equivalent quantity of nitrogen. This factor should be kept in mind in making deductions from this work.

All materials were collected in the fall or in their green stage and dried immediately. Oat straw, rye straw, and corn stover were obtained from farmers north of the college. All other plant materials except cottonseed hulls, buckwheat hulls, sorghum, and seaweed were obtained locally. Only the above ground parts were used.

The materials were prepared by first passing them through an ensilage cutter, which cut them into lengths of a fraction of an inch to more than 1 inch. They were then dried in a steam-heated air drier at 60 to 70°C. After they were thoroughly dried they were ground into a coarse meal in a Hanse Brothers and White burr mill. They were then put into a Wiley mill and chopped until fine enough to pass a 1-mm. sieve. This material was then mixed thoroughly, and a composite sample was taken for analyses. The samples were each placed in a Little Trojan Ball mill and pounded for a day or overnight, which reduced them to a very fine powder, except samples of the monocotyledonous group, which were very hard to powder. The material as taken from the Wiley mill was used for incorporation in the soil.

The organic nitrogenous materials were incorporated in the soil, and the soil was placed in 1-gallon stone jars, moistened to about 20 or 25 per cent moisture on the dry basis, and allowed to set for 1 week.

Total nitrogen determinations were made by the method of Ranker (8). Carbon was determined by combustion in a stream of oxygen, the carbon dioxide being absorbed by ascarite and weighed. Pentosans were determined by the official methods of the Association of Official Agricultural Chemists (1). Lignins were determined by the alkali method of Mehta (7) at 30 pounds pressure, the results being multiplied by 3.743, as 26.72 per cent of the lignocellulose was resolved at this pressure. Nitrates were determined colorimetrically by the use of phenoldisulfonic acid in an alkaline solution (10). Ammonia was determined by the method of Harper (5).

Table 1 shows the nitrogen, carbon, pentosan, and lignin contents of the plant materials; table 2, the ammonia and nitrates liberated in the soil at intervals during decomposition; and table 3, the relative growth of barley plants in sand and in soil to which had been added equal quantities of nitrogen from different plant tissues. Table 4 gives the rates of decomposition of the various plants as determined by the vegetation and by the ammonification and nitrification tests; and table 5, the rates as determined by averaging the results of these two tests. The plants, classified into families, are listed in

TABLE 1

Partial chemical analyses of materials, arranged in the order of decreasing nitrogen content, expressed in percentage of dry weight

MATERIALS	NITROGEN	CARBON	N/C	PENTOSAN	LIGNIN
Pursley	4.48	35.98	1–8	8.90	9.36
Amaranth	3.62	38.01	1–11	9.88	6.66
Cabbage	3.58	41.07	1–12	5.10	4.08
Tomato	3.33	40.89	1–12	7.45	11.34
Tobacco	2.99	38.17	1–13	22.70	4.42
Onion	2.63	41.64	1–15	9.74	4.38
Pepper	2.56	38.25	1–15	8.32	4.23
Bidens	2.55	45.22	1–18	13.30	11.40
Orchard grass	2.54	47.17	1–19	12.14	7.67
Alfalfa	2.43	47.84	1–20	16.93	10.89
Lamb's-quarters	2.43	40.05	1–17	8.62	4.45
Kentucky bluegrass	2.41	46.16	1–19	19.41	1.76
Turnip top	2.32	44.66	1–19	10.98	5.74
Canada bluegrass	2.29	45.84	1–20	13.70	14.15
Knotweed	2.21	40.13	1–18	8.12	10.18
Buttercup	2.17	50.64	1–23	12.51	13.74
Ragweed	2.15	45.16	1–21	14.82	2.06
Xanthium	2.12	44.28	1–21	9.17	6.44
Smartweed	1.94	47.22	1–24	9.27	16.58
Seaweed	1.92	35.88	1–19	10.17	9.55
Red clover	1.76	46.79	1–27	15.53	13.28
Sweet clover	1.67	47.55	1–29	10.59	5.61
Carex lupulinus	1.62	50.69	1–31	23.32	11.34
German millet	1.56	46.19	1–30	26.45	14.84
Juncus effusus	1.52	50.23	1–33	25.54	30.24
Buckwheat	1.50	44.08	1–29	9.76	9.47
Potato top	1.48	35.75	1–25	8.31	10.22
Haircap moss	1.42	43.47	1–27	6.89	16.47
Carex stricta	1.41	50.18	1–35	25.74	21.26
Witch grass	1.36	50.46	1–36	16.96	15.08
Sudan grass	1.36	46.63	1–34	22.91	13.96
Gladiolus	1.21	48.02	1–40	19.73	14.41
Horsetail	1.19	32.89	1–28	6.89	6.40
Fern	1.13	48.71	1–43	7.56	12.76
Fungi	1.09	47.48	1–44	.....	19.24
Oat straw	1.03	49.71	1–48	26.90	18.53
Corn stover	1.02	45.94	1–45	27.17	9.84
Timothy	0.86	49.90	1–58	18.70	13.96
Redtop	0.86	47.68	1–55	21.32	5.20
Club moss	0.83	46.32	1–56	8.82	4.19
Jerusalem artichoke	0.67	45.87	1–68	11.85	3.97
Sorghum	0.62	46.23	1–75	22.06	13.92
Cottonseed hulls	0.57	47.18	1–83	31.63	17.26
Rye straw	0.53	47.20	1–89	27.36	15.16
Buckwheat hulls	0.52	49.76	1–96	24.33	20.62

TABLE 2

Parts per million of ammonia and nitrate nitrogen produced in soil as a result of the addition of 0.41 gm. of nitrogen in the form of plant materials

	7 DAYS		14 DAYS		21 DAYS		28 DAYS		35 DAYS		42 DAYS		49 DAYS		56 DAYS		63 DAYS		AVERAGE WEEKLY	PER CENT OF CONTROL
	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	$NO_3^- + NH_4^+$	$NO_3^- + NH_4^+$
Sudan grass	....	13	1	15	1	30	1	22	6	37	1	54	....	37	....	....	2	34	36	39
German millet	....	25	2	28	1	64	1	48	2	42	1	72	1	69	....	....	1	46	50	55
Oat straw	....	....	1	....	....	T	1	T	4	8	1	14	....	9	....	....	1	23	9	10
Rye straw	....	....	....	....	2	....	....	....	3	5	....	....	2	....	....	....	1	....	2	2
Timothy	....	T	1	....	....	....	....	....	3	....	....	....	2	6	....	....	....	13	4	5
Corn stover	....	....	2	....	....	....	....	T	2	10	3	26	....	2	....	....	2	32	13	14
Red clover	....	33	1	25	2	51	2	82	3	16	1	92	1	62	....	....	1	60	48	52
Alfalfa	....	61	1	81	....	118	....	115	2	75	1	141	....	78	....	....	1	58	91	100
Buckwheat hulls	....	....	....	....	4	....	....	....	2	....	1	T	....	....	....	....	1	....	2	2
Cottonseed hulls	....	....	2	T	1	....	....	....	....	....	....	....	T	1	....	....	....	....	1	1
Sorghum	....	....	....	....	1	....	....	....	2	....	....	....	2	....	....	....	2	....	1	1
Tobacco stems and leaves	....	77	1	97	3	236	....	137	7	117	1	208	1	117	....	....	2	85	136	149
Fungi	....	....	5	....	4	....	8	....	4	T	....	....	5	....	11	....	....	....	6	7
Seaweed	....	....	4	58	4	56	4	57	1	64	3	86	1	87	8	89	....	....	74	81
Club moss	....	....	2	....	3	....	5	....	1	T	2	9	....	5	8	10	....	....	7	7
Horsetail	....	....	3	....	6	13	5	21	2	23	1	46	....	45	10	41	....	....	31	34
Fern	....	....	2	....	2	....	4	....	4	8	1	15	3	15	8	13	....	....	10	11
Buttercup	....	....	3	....	2	9	4	14	7	31	1	30	2	46	7	36	....	....	27	30
Buckwheat	....	....	2	....	2	....	3	....	3	13	1	21	1	22	6	31	....	....	15	17
Knotweed	....	....	3	77	3	58	3	51	3	45	1	73	2	66	5	57	....	....	64	70
Smartweed	....	....	4	....	3	11	2	21	2	36	1	34	4	45	8	53	....	....	32	35
Pepper	....	....	3	23	6	84	4	47	2	47	1	49	1	75	9	79	....	....	61	67

Potato tops	....	....	3	52	2	61	2	70	2	84	3	124	2	141	7	129	....	....	97	107
Tomato tops	....	....	1	48	1	75	3	67	1	83	1	93	3	96	6	99	....	....	82	91
Jerusalem artichoke	....	....	1	....	....	....	2	T	1	....	1	....	5	....	9	....	....	....	3	3
Bidens	....	....	2	27	1	34	1	45	2	41	....	56	2	84	7	99	....	....	57	63
Ragweed	....	....	2	28	1	50	1	43	2	59	1	55	1	64	8	55	....	....	53	58
Xanthium	....	....	....	53	3	37	3	60	3	53	1	83	1	102	8	100	....	....	73	79
Pursley	5	78	6	95	1	101	3	82	3	87	1	78	5	133	....	106	....	....	98	107
Amaranth	6	43	4	85	2	88	2	85	3	88	2	94	....	129	....	93	....	....	91	99
Cabbage	....	26	3	80	2	78	3	76	4	79	....	80	1	118	....	109	....	....	86	94
Onion	4	20	3	37	3	46	3	47	2	49	....	49	1	85	....	71	....	....	52	57
Orchard grass	2	13	5	9	2	12	2	20	3	30	....	30	2	39	3	59	....	....	31	33
Lamb's-quarters	5	14	4	94	2	75	6	100	3	92	2	89	1	133	2	108	....	....	91	100
Kentucky bluegrass	3	34	3	33	5	37	5	45	2	59	1	69	....	46	2	80	....	....	53	58
Turnip top	4	14	4	24	1	22	5	28	3	38	3	37	....	54	3	68	....	....	38	42
Canada bluegrass	1	T	5	27	4	29	3	40	1	60	1	40	1	74	....	66	....	....	44	48
Sweet clover	7	T	5	23	4	34	2	45	1	45	2	57	....	101	3	84	....	....	51	56
Carex lupulinus	....	T	3	....	2	....	3	....	2	....	2	....	....	....	....	10	....	....	4	4
Juncus effusus	....	T	5	....	3	....	4	....	....	....	2	....	1	....	1	....	....	....	2	2
Haircap moss	2	T	3	52	5	31	....	38	3	37	3	38	1	52	1	50	....	....	37	40
Carex stricta	....	....	3	....	1	....	....	....	1	....	2	....	....	....	....	....	....	....	1	1
Witch grass	2	....	3	....	....	....	2	....	3	....	....	....	2	....	5	80	....	....	3	1
Gladiolus	....	....	3	....	1	....	3	....	3	....	4	....	1	....	5	....	....	....	3	3
Redtop	....	....	1	....	....	....	2	....	2	....	3	....	2	....	4	....	....	....	2	3

TABLE 3

Relative growth of barley plants in soil and in sand with and without lime, to which had been added equal quantities of nitrogen from different plant tissues

	PER CENT OF CONTROL		PER CENT OF CONTROL
Soil unlimed:		*Soil limed:*	
Tomato	94	Potato	156
Potato	89	Seaweed	124
Seaweed	87	Tobacco	108
Xanthium	84	Xanthium	96
Knotweed	82	Alfalfa	87
Tobacco	80	Knotweed	85
Ragweed	77	Ragweed	78
Pepper	73	German millet	78
Bidens	70	Pepper	72
Amaranth	62	Bidens	67
Cabbage	61	Tomato	59
Alfalfa	58	Smartweed	58
Pursley	58	Buttercup	57
Canada bluegrass	55	Sudan grass	37
Onion	47	Red clover	33
Kentucky bluegrass	44	Horsetail	33
Buttercup	44	Club moss	28
Horsetail	44	Fungi	20
Turnip top	44	Buckwheat	19
Smartweed	42	Oat straw	19
Sweet clover	40	Fern	18
Orchard grass	40	Buckwheat hulls	15
Haircap moss	35	Corn stover	14
Lamb's-quarters	35	Cottonseed hulls	12
German millet	30	Rye straw	11
Carex lupulinus	25	Jerusalem artichoke	11
Club moss	24	Timothy	10
Buckwheat	22	Sorghum	9
Red clover	21		
Fern	20	*Sand limed:*	
Sudan grass	16	Tobacco	287
Timothy	14	German millet	78
Carex stricta	14	Buckwheat hulls	76
Cottonseed hulls	12	Red clover (dead)	74
Jerusalem artichoke	11	Timothy	67
Oat straw	10	Corn stover	67
Buckwheat hulls	10	Sudan grass	64
Rye straw	9	Alfalfa	58
Fungi	9	Cottonseed hulls	57
Gladiolus	9	Rye straw	57
Corn stover	9	Oat straw	53
Sorghum	8	Sorghum	46
Witch grass	8		
Redtop	8		
Juncus effusus	8		

TABLE 4

Rates of decomposition of plant tissues as determined by vegetation test and by ammonification and nitrification test

Exposed as per cent of control

VEGETATION TEST		AMMONIFICATION AND NITRIFICATION TEST	
1. Tobacco	158.0	1. Tobacco	118.0
2. Potato top	122.0	2. Potato top	109.0
3. Seaweed	105.0	3. Pursley	107.0
4. Xanthium	90.0	4. Tomato	102.0
5. Knotweed	83.0	5. Alfalfa	100.0
6. Ragweed	77.0	6. Lamb's-quarters	100.0
7. Tomato	76.0	7. Amaranth	99.0
8. Pepper	73.0	8. Cabbage	94.0
9. Bidens	69.0	9. Seaweed	82.0
10. Alfalfa	68.0	10. Xanthium	74.0
11. German millet	62.0	11. Knotweed	63.0
12. Amaranth	62.0	12. Pepper	59.0
13. Cabbage	61.0	13. Kentucky bluegrass	58.0
14. Pursley	58.0	14. Onion	57.0
15. Canada bluegrass	55.0	15. Ragweed	57.0
16. Buttercup	51.0	16. Sweet clover	56.0
17. Onion	50.0	17. Bidens	52.0
18. Smartweed	50.0	18. Canada bluegrass	52.0
19. Kentucky bluegrass	47.0	19. German millet	52.0
20. Turnip top	44.0	20. Turnip top	43.0
21. Red clover	43.0	21. Red clover	41.0
22. Sweet clover	41.0	22. Haircap moss	40.0
23. Orchard grass	40.0	23. Sudan grass	38.0
24. Sudan grass	39.0	24. Orchard grass	33.0
25. Horsetail	38.0	25. Smartweed	27.0
26. Haircap moss	35.0	26. Buttercup	24.0
27. Lamb's-quarters	35.0	27. Horsetail	23.0
28. Buckwheat hulls	33.0	28. Oat straw	20.0
29. Timothy	30.0	29. Corn stover	14.0
30. Corn stover	30.0	30. Timothy	13.0
31. Oat straw	27.0	31. Rye straw	13.0
32. Cottonseed hulls	27.0	32. Buckwheat	12.0
33. Rye straw	26.0	33. Sorghum	11.0
34. Club moss	25.0	34. Buckwheat hulls	9.0
35. *Carex lupulinus*	24.0	35. Cottonseed hulls	8.0
36. Sorghum	21.0	36. Fern	7.0
37. Buckwheat	21.0	37. Fungi	7.0
38. Fern	19.0	38. Club moss	6.0
39. Fungi	15.0	39. *Carex lupulinus*	4.0
40. *Carex stricta*	14.0	40. Witch grass	4.0
41. Jerusalem artichoke	11.0	41. Gladiolus	3.0
42. Gladiolus	9.0	42. Redtop	3.0
43. Witch grass	8.0	43. Jerusalem artichoke	3.0
44. Redtop	8.0	44. *Juncus effusus*	2.0
45. *Juncus effusus*	8.0	45. *Carex stricta*	1.0

TABLE 5

*Rates of decomposition of plant tissues, based on average percentages of control from vegetation test and ammonification and nitrification test**

	PER CENT OF CONTROL	N/C		PER CENT OF CONTROL	N/C
1. Tobacco	138	1–13	24. Buttercup	38	1–23
2. Potato	115	1–25	25. Smartweed	38	1–24
3. Seaweed	98	1–19	26. Haircap moss	38	1–27
4. Tomato	89	1–12	27. Orchard grass	37	1–19
5. Alfalfa	84	1–20	28. Horsetail	31	1–28
6. Pursley	83	1–8.	29. Corn stover	22	1–45
7. Xanthium	82	1–21	30. Timothy	22	1–58
8. Amaranth	80	1–11	31. Buckwheat hulls	21	1–96
9. Cabbage	76	1–12	32. Club moss	16	1–56
10. Knotweed	73	1–18	33. Sorghum	16	1–75
11. Lamb's-quarters	67	1–17	34. Buckwheat	16	1–29
12. Ragweed	67	1–21	35. Rye straw	14	1–89
13. Pepper	66	1–15	36. *Carex lupulinus*	14	1–31
14. Bidens	61	1–18	37. Fern	13	1–43
15. German millet	57	1–30	38. Cottonseed hulls	12	1–83
16. Onion	54	1–15	39. Fungi	11	1–44
17. Kentucky bluegrass	52	1–19	40. *Carex stricta*	8	1–35
18. Canada bluegrass	52	1–20	41. Artichoke	7	1–68
19. Sweet clover	49	1–29	42. Witch grass	6	1–36
20. Oat straw	47	1–48	43. Gladiolus	6	1–40
21. Turnip top	43	1–19	44. Redtop	5	1–55
22. Red clover	42	1–27	45. *Juncus effusus*	5	1–33
23. Sudan grass	39	1–34			

* Plant materials arranged in the order of highest percentages of controls from the tests, without regard to family relationship or chemical composition.

TABLE 6

Family grouping of plants, based on percentages of control from nitrification and ammonification test

	PER CENT OF CONTROL	N/C
Solanaceae		
1 Tobacco	118	1–13
2 Potato top	109	1–25
4 Tomato top	102	1–12
12 Pepper top	59	1–15
Leguminosae		
5 Alfalfa	100	1–20
16 Sweet clover	56	1–29
21 Red clover	41	1–27
Compositae		
10 Xanthium	74	1–21
15 Ragweed	57	1–21
17 Bidens	52	1–18
43 Jerusalem artichoke*	3	1–68
Others		
3 Pursley	107	1–8
6 Lamb's-quarters	100	1–17
7 Amaranth	99	1–11
8 Cabbage	94	1–12
20 Turnip top	43	1–19
26 Buttercup	24	1–23
35 Cottonseed hulls	8	1–83
Polygonaceae†		
11 Knotweed	63	1–19
25 Smartweed	27	1–24
32 Buckwheat	12	1–29
34 Buckwheat hulls	9	1–96
Monocotyledoneae—Grasses		
13 Kentucky bluegrass	58	1–19
14 Onion	57	1–15
18 Canada bluegrass	52	1–20
19 German millet	52	1–30
23 Sudan grass	38	1–34
24 Orchard grass	33	1–19
28 Oat straw	20	1–48
29 Corn stover	14	1–45
30 Timothy	13	1–58
31 Rye straw	13	1–89
33 Sorghum	11	1–75
39 *Carex lupulinus*	6	1–31
40 Witch grass	4	1–36
41 Gladiolus	3	1–40
42 Redtop	3	1–55
44 *Juncus effusus*	2	1–33
45 *Carex stricta*	1	1–35
Nonvascular Plants		
9 Seaweed	82	1–19
22 Haircap moss	40	1–27
27 Horsetail	23	1–28
36 Fern	7	1–43
37 Fungi	7	1–44
38 Club moss	6	1–56

* The Jerusalem artichoke sample was not typical of herbaceous tissue; it contained abnormal amounts of stems with ligneous tissue.

† The nitrification rating of Polygonaceae follows the nitrogen-carbon ratio; this was true also of the lower forms of plants.

TABLE 7

Family grouping of plants based on average percentages of control from vegetation and ammonification and nitrification tests

	PER CENT OF CONTROL	N/C
Solanaceae		
1 Tobacco	138	1–13
2 Potato tops	115	1–25
4 Tomato tops	89	1–12
13 Pepper tops	66	1–15
Average	102	
Compositae		
7 Xanthium	82	1–21
12 Ragweed	67	1–21
14 Bidens	61	1–18
41 Artichoke	70	1–68
Average	54	
Leguminosae		
5 Alfalfa	84	1–20
19 Sweet clover	49	1–29
22 Red clover	42	1–27
Average	58	
Polygonaceae		
10 Knotweed	73	1–18
25 Smartweed	38	1–24
31 Buckwheat hulls	21	1–96
34 Buckwheat	16	
Average	37	
Monocotyledoneae		
15 German millet	57	1–30
16 Onion	54	1–15
17 Kentucky bluegrass	52	1–19
18 Canada bluegrass	52	1–20
20 Oat straw	47	1–48
23 Sudan grass	39	1–39
27 Orchard grass	37	1–19
29 Corn stover	22	1–45
30 Timothy	22	1–58
33 Sorghum	16	1–75
34 Rye straw	14	1–89
36 *Carex lupulinus*	13	1–31
40 *Carex stricta*	12	1–30
42 Witch grass	6	1–35
43 Gladiolus	5	1–55
44 Redtop	5	1–55
45 *Juncus effusus*	5	1–33
Average	28	
Others		
6 Pursley	83	1–8
8 Amaranth	80	1–11
9 Cabbage	76	1–12
11 Lamb's-quarters	67	1–17
21 Turnip top	43	1–19
24 Buttercup	38	1–23
38 Cottonseed hulls	12	1–83
Average	57	
Nonvascular plants		
3 Seaweed	98	1–19
26 Haircap moss	38	1–27
28 Horsetail	31	1–28
32 Club moss	16	1–56
37 Fern	13	1–43
39 Fungi	11	1–44
Average	34	

table 6 with the percentages obtained in the ammonification and nitrification tests, and in tables 7 with the percentages obtained by averaging the results of the vegetation and the ammonification and nitrification tests.

DISCUSSION

The following questions were in mind when this experiment was planned:

Is there a relationship between the characteristics (such as reproductive mechanisms) by which plants are at present classified into families and the rate of decomposition when they are incorporated in the soil?

Is there a relationship between the total nitrogen content of plants and the rate of decomposition?

Is there a relationship between the carbon-nitrogen ratio and the rate of decomposition?

Is there a relationship between the lignin content of plants and the rate of decomposition?

Is there a relationship between the content of pentosans and the rate of decomposition?

Is there a relationship between the rate of decomposition and the characteristics of plants (such as their position in the evolutionary series) by which they are sometimes classified into "natural" families?

The results of this experiment also open up many other interesting questions, for the solution of which the data obtained are insufficient.

Tables 6 and 7 show how, under the conditions of moisture, temperature, and preparation in this experiment, plants of the same family tend to group themselves with respect to readiness of decomposition. Table 2 shows in a little more detail the weekly fluctuations in the amounts of nitrate and ammonia liberated by these individual members. Individuals of the same family observed under the conditions of this experiment and cut down under similar conditions of growth and at similar stages of development break down similarly. The smaller the number of representatives used the narrower is the range of fluctuations; and, as a study of the monocotyledonous group shows, the larger the number, the wider is the range of fluctuations. There is, consequently, a narrower range of fluctuations among members of families in the dicotyledonous group than among members of the monocotyledonous group of plants. This relative uniformity may be the result of the fact that fewer representatives of the dicotyledons were observed, that the conditions under which the members of this family grew were more nearly similar, or that, because the dicotyledons decayed more rapidly, their decomposition was either less complex or less accurately recorded. Certain members within families also show similarities in behavior. Thus the bluegrasses, sudan grass, orchard grass, and onions, all of which are calciphiles, all broke down rapidly, but other calciphiles such as sorghum and timothy, required more than 8 weeks for complete disintegration.

There is a positive correlation of 0.81 ± 0.035 between the rate of decomposition as determined by ammonification tests and the total nitrogen-content of the plants studied. The larger the percentage of total nitrogen, the sooner the plant (when finely ground and incorporated in the soil) begins to liberate and add its nitrates to those of the original soil, and the more rapid thereafter is the rate of liberation. But when the total nitrogen is more than 1.50 per

cent in dicotyledonous plants, and more than 2 per cent in monocotyledonous plants, nitrates should appear from them in the soil within approximately 14 days. Little if any correlation was found, however, between the total nitrogen content of the members of families of plants and their position in the usual classification, but a surprisingly close (probably fortuitous) relationship existed between the three members of the Compositae studied and their total nitrogen content.

Some relationship was found between the groupings of plants into families and the carbon content of the separate members. This relationship was closest among the dicotyledons, but appeared to be a more or less constant factor for all the plants studied. The largest percentages of carbon were found in the monocotyledonous plants. It seems clear to the writer that a high nitrogen content is seldom associated with a high carbon content (indeed, a negative correlation of 0.53 ± 0.07 between the two was found) and as the carbon content increased, the nitrogen content usually decreased. The larger the carbon content the slower was the rate of decomposition, for a low content of nitrogen in an organic fertilizer always results in a nitrogen depression in the soil, and, of course a temporary falling off in the yield of crops.

Under the conditions of this experiment the carbon-nitrogen ratio cannot be relied upon to predict which of two plant materials or organic substances will be nitrified the more readily. The writer found that plant tissue with a nitrogen-carbon ratio of 1 to 25–30 was very readily decomposed to yield up its nitrates. As the ratio decreases from 1 to 25–30 to 1 to 8 (the normal ratio of nitrogen to carbon in the soils) the balance of organisms in the soil is less violently disturbed, and the nitrates given up in decomposition soon appear. The nitrogen-carbon ratio can be used, however, to predict at times the relative rapidity at which nitrates given up in decomposition may be expected to appear in the soil, or, vice versa, the relative nitrogen depression, generally speaking, of crops. Tables 4 and 5 show these relationships.

It will have been observed by the reader that the lignin content of plants is a large contributor to humus. In its process of decay, humus at first noticeably deteriorates, but it soon reaches an equilibrium, after which decomposition is so slow that lignin materials tend to accumulate, and the relative proportion of lignin in the soil to increase. Thus, there was a negative correlation of 0.357 ± 0.08 between the lignin content of plants and the parts per million of nitrates formed. The higher the lignin content, too, the slower is the rate of decomposition; for the lignin as ligno-cellulose tends to shield the cellulose from decomposition by soil organisms. Waksman (11) has shown, in fact, that plant tissue from which the lignin content has been removed decays readily.

So far as the rate of decomposition is concerned, a negative correlation of 0.474 ± 0.02 was found between the results of the nitrification test and the percentage of pentosans in plants; in other words, as the pentosans increased, nitrification decreased. The results are remarkably and significantly similar

to those of the carbon and nitrogen tests. There was, on the other hand, a positive correlation of 0.450 ± 0.08 between the content of pentosans and the content of lignin. These are precisely the results one would expect, and the two tests seem to substantiate each other. In short, as the total nitrogen content decreased, the percentage of pentosans increased.

In order to clarify further applications of the results of this experiment to the "natural" classification of plants, it will be necessary to state one assumption on which all such classifications depend. It is assumed by students of botanical (and other) evolution that various forms have developed on the earth at different rates over the same period of time, for only by such an assumption, apparently, is it possible to explain the contemporaneous presence of simple, unadapted forms, on the one hand, and complex, highly specialized forms, on the other. Thus, certain plants were at some older epoch forced so to adapt themselves to their environment as to evolve a very high tolerance for nitrogen, whereas others, living in the same epoch but presumably under other conditions, escaped this necessity altogether. Today, therefore, the one species contains a relatively large proportion of nitrogen, and the others, a relatively small proportion. It seems evident, if the original assumption is granted, that a corollary of it must also be true that the very existence of the first species proves the existence at an earlier epoch of an environment which somehow forced nitrogen upon plants in unusual amounts. If this original assumption and its corollary be admitted, then this experiment shows that as the dicotyledons were developing as a distinct order, there must have been what may be called for the sake of brevity a *nitrogenous environment* and that this nitrogenous environment must have faded before the evolution of the monocotyledons, which contain less nitrogen. (It may be required, indeed, whether there was not some sort of correlation between the "nitrogenous environment" and the evolution of the two seed leaves of the dicotyledons.) Thus, such low forms as the lichens, the horsetails, and the club mosses contain little nitrogen and (at least the last two) much silica, but a dicotyledon like portulaca is rich in nitrogen. With a monocotyledon, like rye, however, the total nitrogen content is exceedingly small.

The mention of the proportion of silica in plants leads to a still further development of the theory of environments at older epochs which were characterized by a predominance of one or a few elements or compounds. It has just been remarked that the "nitrogenous environment" in which the dicotyledons appear to have evolved had faded before the monocotyledons were developed. It will be recalled, too, that as the content of nitrogen decreased, that of the pentosans roughly increased. It seems to follow conversely that if a steady increase in the proportion of the pentosans in the higher members of the evolutionary series can be shown, it is safe to assume (even without analytical proof) that the proportion of nitrogen must be steadily declining, indeed, that the "nitrogenous environment" is waning. The ratios of percentages of silica to percentages of nitrogen (table 8) indicate exactly the same

recession of nitrogen before the advance of another, opposed, constituent of plant tissue. If, then (as seems likely from the foregoing comparison of nitrogenous content with position in the evolutionary series) "nitrogenous environments" come and go in cycles, the steady increase of the proportions of silica indicate that the botanical world is now in the downward curve of a cycle. Thus, the proportion of silica in the plants at the bottom of the evolutionary series is negligible; it increases in the higher order of Pteridophytes (such as the horsetails); it decreases, however, in the still higher order of dicotyledons;

TABLE 8

*Proportions of silica and lime in monocotyledons and dicotyledons**

	CaO	Si		CaO	Si	
	per cent	*per cent*		*per cent*	*per cent*	
Alfalfa	1.92	0.065	Kentucky bluegrass	0.264	2.72	
			Canada bluegrass	0.654	2.72	
Beans	....	0.820	Broom sedge	.....	2.28	
			Corn plant	0.590	1.10	
Clover	2.90	0.238	Oat plant	0.418	2.10	
			Orchard grass	0.412	4.40	
Peas	....	0.250	Timothy	0.285	0.784	
			Wheat	.....	2.50	
Beets	....	0.014	Rye	0.430	.....	
Cabbage	2.42	0.184	Average	0.44	2.325	
Cotton plant	....	0.290				
Lettuce	....	0.538	Fern	1.01	0.16	
Potato vines (sweet)	....	0.410	Horsetail	2.30	3.65	
Potato tops (white)	1.72	.....	Club moss	0.27	0.31	1.07
Tobacco	4.42	0.125	Sphagnum	0.29	0.16	
Tomato	2.77	0.519	Marchantia	0.94	0.09	
Lamb's-quarters	2.93	0.200	Fucus	1.62	0.03	
Buttercup	1.34	0.200	Lamnaria	1.58	0.14	
Sunflower	2.45	1.160	Fungi	0.03	0.04	0.08
Turnip top	3.55	0.060	Average	0.88	0.56	
Average	2.56	0.34				

* Compiled from analyses made upon ashes of the plants by Honcamp (6, p. 180–220), Wolff (12), and Robinson (9).

and gains once more in the monocotyledons, the highest of the orders included in the experiment.

In table 8 it is clear that there is a positive (though rough) correlation between the proportions of lime and nitrogen in plants. Although no information has yet been collected to show precisely what the circumstances are that force plants to tolerate more nitrogen (whether the cause relates to climatic conditions, nature of the soil, species of plants or stage of growth, or a combination of these), yet tentative data *have* been submitted regarding the circumstances

which favor the incorporation of lime in plants. It may be assumed that there is some correlation between the two: that conditions which favor a high tolerance of nitrogen are not unlike those which are fairly certain to favor a high tolerance of lime. The conditions which favor a high tolerance of lime relate principally (as far as is known) to the nature of the soil or to climatic conditions. The lower orders of marine plants—the fucus and lamnaria, for example, of which the absorptive organs subsist in submarine soil, if any, usually contain proportionately more lime than do the lower orders of terraneous plants. Moreover, heavy rainfall (because it dissolves much lime from limestone deposits) renders unusually large amounts of the compound available to plants, and excessive heat (by forcing upon plants the defense of rapid transpiration) makes for the absorption of unusually large amounts of the water in which alone a plant can receive lime. From these tentative principles, at least this much may be hazarded about the "nitrogenous environment" in which the dicotyledons evolved: that it was a more moist, probably warmer period than that in which the later monocotyledons developed, for the average percentage of lime in the former order is 2.56, and in the latter, only 0.44. It will be clear, too, that an analysis of the relative proportions of lime in plants also substantiates the "natural" classification.

Although the writer feels that the method which he used to determine the lignin content of plants has not yet been perfected and would not serve, for example, in the analysis of a wide range of species, yet the figures for lignin content obtained by the imperfect method used are of considerable use and still further substantiate the various "natural" classification of plants. The lignin content of the monocotyledons is higher on the average (4 per cent) than that of the dicotyledons (2.50 per cent); and, correspondingly, the former stand higher in the evolutionary series than the latter.

The correlations between proportions of pentosans in plants and their membership in families are not so definite. To be sure, the proportion of pentosans in the grasses range from 12 per cent in orchard grass to 27 per cent in rye straw. In the nightshade family the proportion of pentosans in three out of four specimens were very similar (7–8 per cent), but tobacco (also a member of the nightshade group) contains 23 per cent of pentosans. In the polygonum (without seeds and hulls) the percentage of pentosans ranges from 9 to 15 per cent. In the leguminosae, the range is from 11 to 17 per cent. On the other hand, the dicotyledons are, as a rule, poor in pentosans, the average for the group being but 12 per cent; and the monocotyledons were the richest of all the plants tested, their average being 22 per cent.

Judging from the percentages of pentosans in their makeup alone, one could assume that the monocotyledons are higher than the dicotyledons in the evolutionary series of plants. Indeed, a family tree could be worked out on the basis of the proportion of pentosans in plants. On such a tree, the plants would appear in the following order (from the highest to the lowest): The *Monocotyledons*—rye straw, oat straw, and corn stover, followed by the carices,

Juncaceae, sorghum, redtop, and the bluegrasses, which serve as a link connecting the monocotyledons with the dicotyledons that follow; the *Dicotyledons* —the Compositae (with 12 per cent of pentosans), the Leguminosae (11 per cent), the Polygonaceae (9 per cent), and the Solanaceae (8 per cent). Intermediate in the arrangement of the dicotyledons would fall the Cruciferae, Amaranthaceae, Portulacaceae, and Chenopodiaceae. Since only one or two specimens of the families were studied, these groups can be placed only conjecturally and approximately. The lower forms of terraneous plants would fall beneath the Solanaceae on the family tree and would lead at their lower extremity to the algae.

The numbers of different plants used are not sufficient to justify a statement involving generalizations, but the conclusions may be of such a nature as to suggest probabilities as to reasons of behavior of certain plant tissues when subjected to agencies of decomposition.

SUMMARY

A partial chemical analysis (including determinations of the proportions of nitrogen, carbon, lignin, and pentosans) has been made of 43 different plants and 2 seed parts, the majority of which plants are common weeds or widely cultivated crops.

The decomposition of these plants has been studied in the greenhouse in cultures of four different kinds: soil without lime, soil with lime, sand without lime, and sand with lime. The criteria for the rate of decomposition were the amounts of ammonia and nitrates formed.

The plant tissue incorporated in these cultures for the purpose of the experiment were all collected in as nearly comparable stages of growth as possible but were obtained from a wide variety of soils and locations.

It was found that the average proportion of nitrogen was greater in the dicotyledonous than in the monocotyledonous group, ranging in the former from 0.67 per cent in the Jerusalem artichoke to 4.48 per cent in pursley, and in the latter from 0.53 per cent in rye straw to 2.54 per cent in orchard grass.

The content of pentosans ranged in the dicotyledons from 5 per cent in cabbage to 23 per cent in tobacco, and in the monocotyledons from 12 per cent in orchard grass to 27 per cent in rye straw. As the nitrogenous content decreased, the content of pentosans increased.

The proportion of carbon was highest in the monocotyledonous group, ranging from 42 per cent in the onion to 50 per cent in witch grass. In the dicotyledonous group it ranged from 36 per cent in potato tops to 48 per cent in alfalfa, increasing with the content of pentosans.

As the proportion of carbon and pentosans decreased, the rate of decomposition (as shown by the amounts of ammonia and nitrates formed, and by the increased plant growth) increased.

There is not, apparently, a strict correlation between the rate of decomposition and the total nitrogen content of plants or the nitrogen-carbon ratio.

Yet the experiment shows very clearly that, on the whole, the plants containing most nitrogen decompose more readily than do those containing less. The structure of plants and their content of lignin and pentosans (which resist both grinding and decomposition) were also factors in the rate of decomposition, for plants of the dicotyledonous group were more easily ground than plants of the monocotyledonous group and were less resistant to decomposition. If the ratio of nitrogen to carbon is no more than 1 to 25–30 the plants decompose easily, but if the ratio is wider, the incorporation of natural fertilizers in the soil results in a depression of the nitrates.

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THE TRIPLE-ANALYSIS METHOD OF TESTING SOIL FERTILITY AND PROBABLE CROP REACTION TO FERTILIZATION

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The determination of the availability of plant nutrients in the soil is an important problem of applied soil science. The farmer wants to know the value of his soil as a substrate for crop growth, and he has a need of quick methods that will reveal the extent to which his soil must be fertilized. To solve these problems, agricultural science has used both field and laboratory experiments.

The field experiment is used the world over as the practical test of soil fertility. This test is very little suited for generalization, however, because the causal relationships between the amounts of mineral fertilizers added and the weight of the harvested crop are still unknown; moreover, the field experiment is greatly affected by climatic conditions, which vary in an unforeseen manner from year to year. Furthermore, as a test for farmers, the field experiment is too expensive and laborious.

From the standpoint of soil science and plant physiology it seems futile to attempt to develop a single method of testing the productivity of a soil under all circumstances. Varying climatic conditions, as well as the heterogeneity of the soil, will militate against any attempt to find a general test. Plant physiology and soil science together will nevertheless be able to supply some information on the reaction of individual soils to fertilization and on the average suitability of a particular soil to a particular crop. Work is being done along these lines, but full information is not yet available. This paper is limited, therefore, to the fundamentals involved in such experimentation and to an account of a method developed in this laboratory.

PHYSIOLOGICAL BASIS OF THE METHOD

Experiments on the mineral nutrition of different plants, performed in pot cultures and in water cultures, show the relation between the concentration of the nutrient ions, on the one hand, and plant growth and development, on the other. These relations are not simple, unless single monovalent ions such as K^+ and NO_3^- are varied. In this case, more or less regular optimum curves generally arise. But there is also an interaction between different ions, known as "ion antagonism," that might change markedly the type of the growth curves. Such antagonistic phenomena occur between K and Ca, Mg and Ca, and Ca and Mn. In Scandinavian soils the antagonisms $K \rightleftarrows Ca$ and $Ca \rightleftarrows Mn$

predominate, but in soils of other kinds such antagonisms as K⇄Na and Ca⇄Mg may also have a practical bearing. Recent researches (2) have shown that the antagonistic action of one ion upon another is chiefly a function of the ratio of molar concentrations. If $M_1:M_2>1$, the ion M_1 will suppress the entrance of M_2 into the plant; if $M_1:M_2<1$, the reverse will be true. If the concentration of the different ions in the nutrient solution is known, it will be possible to determine, within a certain limit of probability, whether antagonistic effects will occur. This statement is based on the supposition, of course, that the reaction of the plant has been tested by experiment, because such factors as the higher mobility of K^+ in comparison to Ca^{++} modify the balance within the plant (6, 9). This is the physiological basis of the method. In the following discussion plant growth is considered as representative of the size of the harvest, without regard to detailed relationships between straw and grain, which by some extension of the scheme might be included in the test.

The need of a plant for mineral nutrients and the reaction of the plant to varying proportions and concentrations of these nutrients can be demonstrated in water cultures. But to what extent it is possible to apply the results of water cultures to field conditions is still an open question. The mechanism of absorption is undoubtedly the same in solutions and in soil—this has been demonstrated, for instance, with respect to ion antagonism (6, 7)—, but the content of colloids influences considerably the absorption velocity. Such factors as mobility of ions in colloids and exchange of ions between colloids (root colloids and soil colloids) interfere with absorption. Moreover, the solubility of phosphates and carbonates in relation to cH plays a dominant rôle in solid substrates. If growth tests have to be made, samples of the soil in question must be used in pot experiments. This method, however, is laborious and expensive, and its use, like that of the field experiment, is limited by our ignorance of such factors as the laws of uptake and distribution of ions, ion antagonism, and relation between inner concentration of elements and growth. In the author's opinion, a good practical test of the fertility of the soil and its fertilizer requirement must be built up from scientific knowledge of the soil and its influence on the growth of the plant.

EXPERIMENTAL

In a series of nutrition experiments on oats grown in quartz sand moistened with mineral solutions, performed in this laboratory, not only the growth was determined but also the content of different nutritive elements (K, Ca, Mg, Mn, P) in the green parts (leaves and straw). In calculating the concentration of elements in the dry matter (millimoles per kilogram) certain relations to growth were found. The ash analysis also revealed relations between absorption and concentration of the ions in the substrate. The idea thus arose of using the results of ash analyses of the green parts as a test for the available nutritive elements in the soil and for the properties of the soil as a growth substrate.

This idea is perhaps not new, but since a cheap and satisfactory method of chemical analysis—quantitative spectral analysis (8)—has been devised, it seems to be worthy of test on a large scale. Some preliminary work has already been done, but a thorough investigation will take years. The outlines of this reserach work are sketched in the following pages.

The first question to be considered is the relation between the growth of the plant and the concentration of the nutritive elements in the plant. The roots are excluded from analysis, as far as field plants are concerned. It is a well-known fact that the composition of the seed ash varies very little with the nutrition (6). If roots, straw, and leaves of cereals are separately analyzed, the results show a marked difference between the distribution of such elements as K and Ca (2, 6). This question has been studied in detail in this laboratory by Burström (2, 3), who showed that in studying the physiology of absorption, all parts of the plant must be considered and that the proportion of the different elements (e.g., K:Ca) varies widely in different organs when the nutrient

TABLE 1

The normal and the minimum content of elements in leaves of oats from Sweden, compared with the content of the same elements in citric-acid extracts of the soil

ELEMENT	MILLIMOLES PER KGM. DRY MATTER		MILLIMOLES PER KGM. SOIL
	Normal	Minimum	
Na	40–100	...	1–2
K	200–400	100	0.5–1.0
Ca	180–250	45	50–100
Mg	100	?	4–10
P	75–100	75	1–2

solution is varied. If the problem is to determine the uptake of a particular element under different conditions, however, it seems to be sufficient to know the concentration of the element in the leaves (3, p. 8).

The concentration of the mineral nutrients in the plant is regulated partly by the absorption intensity of the roots and partly by the consumption during growth. If growth and consumption run parallel, the inner concentration will remain constant. If growth proceeds more slowly than does the rise of inner concentration, a higher concentration will indicate a more intense growth. It is evident that only in the latter case will the determination of the ash contents of the leaves be of any use for the estimation of growth intensity. Our experiments show that the inner concentration of potassium in the leaves of oats within a wide range reflects the growth. The inner concentration of calcium also reflects the growth, but the region of positive influence is narrower. For both these ions, a distinct minimum exists below which no growth occurs; the maximum covers a wider range, but undoubtedly an upper limit exists above which an injurious effect is noticed.

There is no marked relationship between a variation of the inner P-concentration and growth. Nevertheless, a minimum concentration exists, below which growth is retarded. The estimation of the P-concentration is therefore of great value in revealing deficiency of phosphorus in the growth medium. Whether nitrogen might properly be included in the test is rather doubtful. This element takes part in so many chemical processes in the plant that it seems illogical to consider "concentration" of nitrogen in the same category with concentration of the mineral ions. Phosphorous also takes part in a great number of chemical reactions, but for the most part, maintains its character as an acid.

For a successful use of the ash analysis as a growth test it is necessary to know something about the best time of sampling. Burström (3) analyzed oat leaves in different stages of development and concluded that as far as the cations (K, Ca, Mg, Fe, Mn) are concerned the leaves should be collected green after the setting of the ears but before the ripening of the flowers [see also (7)]. This is in agreement with the results of Petrie (10) on Lolium and of Zattler (12) on hemp. Both of these investigators found a continous absorption of minerals during the development of the plant. In many plants the absorption is retarded, however, before the full development of the flowers, and it seems advisable therefore, to choose an earlier time for sampling. Further investigation of this question is necessary. The influence of phosphorous absorption on growth seems to be restricted to the early stages of plant development (4, 11, 12). The P-content of mature leaves, therefore, varies more widely than, for example, the K-content. Of practical importance is the statement of Burström (3) that the samples of the leaves must be carefully collected and that the leaves must be cut off from the sheaths.

The applicability of the complete ash analysis and the determination of the "dry concentration" (millimoles per kilogram dry substance) of the elements has been tested in a number of field experiments, chiefly with oats (3, 7, 9). Table 1 shows the normal and the minimum content of elements in oat leaves and the content of the same elements in citric-acid extracts of soil, as determined by Lundegårdh (7). The normal content of potassium in oat leaves in Swedish fields was 200–400 millimoles per kilogram dry weight, and that of calcium was 180–200 millimoles. On some light soils in Västergötland in South Sweden the leaves contained 310 millimoles Ca and only 76 millimoles K. The conclusion seemed justified that potassium fertilization would pay. The addition of 150 kgm. 40 per cent K-salt raised the inner K-concentration to 294 millimoles, and a rise in seed-weight of 51 per cent was observed. On another field the leaves contained 308 millimoles potassium and 171 millimoles calcium. The effect of potassium fertilization here was consequently small. Burström (3) studied the effect of fertilization on some fields where the leaves contained as much as 449–880 millimoles of K per kilogram and only 117–136 of Ca. On these fields, potassium fertilization showed a negative effect. The concentration of phosphorous was very low, only 47–63 millimoles, whereas a

minimum of 75 was calculated. The effect of phosphorous fertilization was consequently positive.

Much wider experience is needed, of course, before any definite conclusions can be drawn as to the reliability of the "dry concentration" of mineral elements in the crop as a test for the nutrition situation in the soil, but the preliminary results seem to be encouraging. The research work to be done is primarily the estimation of the "concentration curves" of the growth of different crops. The fundamental thesis here is that *growth is directly related to the inner concentration of the mineral elements*. Certain minimum and maximum concentrations have already been determined, and for some elements (especially potassium) closer quantitative relations to growth evidently exist. Probably certain tolerable and intolerable concentrations as well as injurious and favorable proportions of the elements exist; for example, the ratio K: Ca seems to have an upper and a lower limit beyond which growth is hindered.

From the foregoing discussion, one might erroneously conclude that a "single" analysis of the leaves is sufficient for the determination of soil fertility. Up to this point we have limited ourselves to establishing the correct physiological basis for such a determination; namely, growth is primarily determined by the distribution and concentration of the nutrients within the plant and is only secondarily dependent on the concentration of the nutrients in the soil. This is very important, for the quantity of an element (ion) absorbed is not generally in direct proportion to the outer concentration (2, 6). We shall not go into detail here concerning the absorbing power of the plant. This has been discussed in previous papers (7, 9), in which, also, the relation between the concentration of elements in soil extracts and in the leaves has been demonstrated.

In some instances the ash analysis alone gives enough information; in most instances ash analysis plus analysis of the surface layer of the soil will be sufficient; and in still other instances ash analysis, analysis of the surface soil, and analysis of the subsoil are needed. The three analyses represent a real "triple analysis," but this term is proposed as a general name for the combined chemical-physiological test of the soil.

In the triple-analysis method, the value of the analysis of soil extracts depends upon the method of extraction and the completeness of the analysis. Unfortunately, no chemical extraction medium is able to duplicate the absorption process of living roots. The experience of this laboratory has demonstrated, however, that weak acids, such as citric acid, give a fairly good picture of the quantity of available alkaline substances in the soil (7, 9) and sufficiently accurate values for Ca and Mg to warrant conclusions on the antagonistic effects of these ions. In a previous paper (9, p. 99, fig. 5) the markedly antagonistic effect of Ca in the soil on the absorption of Mn by sugar beets is demonstrated. The citric-acid extract, however, gives no convincing results with phosphorus, because no close relation was found between the P-content of the soil and the absorption of the plant. The solubility of the phosphorus

compounds is dependent to a great extent on the cH of the medium, and different extraction agents (citric acid, lactic acid) therefore will give different values. The phosphorus question, furthermore, is complicated by the fact that the intake of P in the plant is influenced by such factors as the lime content of the soil (3, p. 27). The extraction methods also give some dubious results with regard to the solubility of Mn, but for the estimation of available Mn the pH of the soil and its Ca content are far more illuminating than are the absolute quantities of Mn (6, 7, 9). As the pH of the soil controls the solubility (availability) of several elements (Ca, Mg, Mn, Fe, Cu) the determination of the factor cH is a useful complement to the determination of the metallic elements, but the importance of this factor should not be overestimated in comparison to that of other ions.

In spite of the fact, previously mentioned, that the extraction methods give no reliable picture of the availability of the mineral nutrients, the complete analysis of soil extracts made with a weak acid plus the pH determination should constitute a necessary complement to the complete ash analysis. A marked parallelism exists, for example, between the potassium content of the soil extract and the potassium content of the plant (7, 9). Swedish soils (from about 300 places) show an average potassium content of 1 millimole per kilogram and a calcium content of 50 millimoles (table 1). Pot and field experiments show that under these circumstances no appreciable antagonistic effect of potassium upon the uptake of calcium exists. On the contrary, liming tends to inhibit the uptake of potassium and also of the heavy metals—Mn, Fe, Cu (3). A preliminary estimation of the balance or lack of balance between K and Ca can be made from the soil analysis, considered in the light of broad experience in the behavior of different soil types.

Experiments show that the majority of mineral nutrients are absorbed from the surface layer of the soil ("Ackerkrume"), whereas the subsoil is of greater importance as a source of water supply. In exceptional cases, however, the subsoil contains more nutrients than does the surface layer (7, 9) and consequently furnishes the plant with salts. The physical properties of the subsoil, its content of colloids, especially iron compounds, and the thickness of the surface layer seem also to have an influence on the development of the plant.

CONCLUSIONS

A combined chemical-physiological test of the fertility of the soil and the probable reaction of a crop to fertilization has been discussed.

The full test involves three complete analyses as follows: analysis of the green leaves of the crop, collected before florification; analysis of the surface soil (citric-acid extract of a dry sample taken at a depth of about 15 cm.); analysis of the subsoil (citric-acid extract of a sample taken just below the upper limit of the subsurface layer). The subsoil sample may be omitted in many tests. The sampling of the soil is, of course, subject to modifications according to soil

types. The directions mentioned refer to Swedish agricultural soils. The analysis comprises that of the following ions: H, K, Na, Ca, Mg, Mn, Fe, Cu, and PO_4. For comparison and control, Li and Rb, which are normal constituents of the soil, might also be included. Nitrogen nutrition is not included in the test.

The fundamental technic of the test is quantitative spectral analysis, which is a very rapid, cheap, and exact method of cation analysis. For pH and PO_4 determinations, common electrometrical or colorimetrical methods are used.

The physiological bases of the triple-analysis test are the facts that growth and development of the plants are primarily dependent upon the inner concentration and distribution of the nutritive elements (ions) and that the ash of the leaves, within certain limits, reflects the nutrition condition of the whole plant. Growth ceases when the concentration of any of a number of elements (K, Ca, P, and probably Mn and Fe) in the leaves falls below a minimum, and for some elements, especially potassium, quantitative relations are perceptible. The leaf sample will show whether one element is lacking from the soil and also, to a certain extent, whether an injurious lack of balance exists between two or more elements, for example between K and Ca.

The soil sample shows directly the minimum availabilities of alkaline substances and the probable inhibiting effect of lime on the uptake of manganese and potassium, or the antagonistic effect of potassium on calcium.

The practical application of the triple-analysis test has been studied in regard to potassium fertilization, liming, and manganese-deficiency diseases. Further accomplishments of the test depend upon the carrying through, on a large scale, of combined laboratory and field experiments. The physiological reaction of different agricultural plants must be studied (hitherto only oats and, to some extent, sugar beets have been investigated). We need more information on the reliability of the leaf test. For some plants it might be necessary to use leaves in a special stage of development or even to use other parts of the plant (straw, stem, or root sap). Also the preparation for analysis needs attention. Instead of heat-ashing, which is laborious and may result in loss through volatilization, wet-ashing or even the sap obtained by hydraulic pressure might be used. Due attention should be paid to the statement of Arens (1) and of Lausberg (5) that certain elements (K, P) form on the upper surface of the leaves an exudate that is washed off by rain. The suitable preparation of the soil for analysis ought to be studied in conjunction with pot experiments. Perhaps a good method for the determination of the soil colloids or the amount of exchangeable ions would also be profitable. The basis of the triple-analysis method, however, is the substitution of the leaf test for a more accurate—and chemically unattainable—extraction procedure. The triple-analysis method uses the plant itself as the extraction agent, whereas the soil analysis reveals the general characteristics of the soil. Nevertheless, even in this respect, possible progress in the manner of preparation should not be neglected.

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FACTORS INFLUENCING THE OCCURRENCE OF AZOTOBACTER IN IOWA SOILS[1]

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The discovery of the nitrogen-fixing bacteria of the genus *Azotobacter* by Beijerinck in 1901 has led to many researches dealing with the distribution of these bacteria in soils and with their economic importance in the maintenance of soil fertility. Much information has been obtained, but many observations are still unconfirmed, and some questions are not yet answered.

Numerous factors have been found to influence the growth and activity of the Azotobacter. Among the most important is that of the soil acidity. It has been claimed (8, 9) that the ability of a soil to support the growth of these organisms can be controlled at will by artificially adjusting the reaction, the limiting reaction being placed at pH 6.0. Vandecaveye and Anderson (16), however, found the bacteria present in some western Washington soils which were more acid than pH 6.0, and Wilson, et al. (17, 18) were able to induce the development of macroscopic Azotobacter colonies upon the surface of soil plaques in all of a series of New York soils tested, regardless of acidity, if the proper salt plus a carbohydrate mixture was incorporated with the soil. It was pointed out by these investigators, therefore, that other factors in the soil environment may be more important in controlling the growth of the Azotobacter than is the soil reaction.

Burk and co-workers (5), however, in exhaustive tests with pure cultures of Azotobacter checked Gainey's work and showed conclusively that a pH of about 6.0 or lower is limiting for the growth of the Azotobacter in the absence of fixed nitrogen. When fixed nitrogen was present in the medium, the bacteria were able to thrive at reactions far below pH 6.0. This latter finding may offer an explanation for the results of Vandecaveye and Wilson and their coworkers.

Winogradsky (21) points out that caution should be exercised in applying the results of pure culture work to the soil. He found that the effect of fixed-nitrogen additions to the soil was to depress and, in many soils, entirely to

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eliminate the Azotobacter. Winters (22) found that small amounts of nitrogen added to the soil stimulated the growth of the Azotobacter but that heavier applications depressed it

Niklas, et al. (14) attributed their failure to find Azotobacter in some soils with favorable reactions and high lime contents to a lack of phosphoric acid in the soil. Ziemiecka (23) found that the lack of soluble phosphate in some Polish soils did not affect the presence of the Azotobacter but did stop their activity. Burk and Lineweaver (4) found phosphorus, except in very small concentrations, to be nonessential to the growth of pure cultures of the bacteria. At higher concentrations, however, it stimulated their growth.

It is evident, therefore, that not all the investigators are agreed on the factors which influence the presence of the Azotobacter in soil. As a consequence, further investigation of this problem is necessary to clear up some of the questions which must be answered if the growth of these organisms in all soils is to be obtained and especially if successful soil inoculation with the organisms is to be accomplished when necessary.

A previous investigation of the occurrence and distribution of Azotobacter in Iowa soils (12, 13) showed that many of the more important soil types of the state, from the standpoint of total acreage, were virtually devoid of the bacteria. Correlation studies of the chemical composition of the samples collected and the presence of the Azotobacter indicated that the high acidity of the large majority of these soils was the most important factor limiting the presence of the bacteria and that the available phosphate content may also be of importance in many cases. Other factors being favorable, the results also indicated that the amount of growth which the Azotobacter would make in the soil depended largely upon the organic matter content and upon the pH.

The present investigation was planned as a continuation of the study of the factors which influence the presence of the Azotobacter in Iowa soils. It was thought desirable to check the results of the correlation studies with more controlled experiments in which the effect of various soil treatments upon the growth and activity of the Azotobacter while in the soil would be quantitatively determined.

EXPERIMENTAL

The results which were obtained with typical samples of two soil types, Grundy and Clinton silt loam, are presented in this paper. Other types were also used, but the results were essentially the same as those given. Grundy silt loam was very acid in reaction (pH = 5.17) and had a lime requirement of approximately 4 tons an acre (based on 2,000,000 pounds of surface soil per acre). It represents a soil type developed under prairie conditions, and it contained, consequently, a large amount of organic matter. It is of loessial origin. Clinton silt loam is also of loessial origin, but it developed under forested rather than prairie conditions and, as a consequence, contained very little organic matter. It was less acid in reaction than Grundy silt loam, having a pH of 6.22 and a lime requirement of about 2 tons an acre. Both of

these samples were alike in that they represented soil types which occur extensively in the state and which are generally lacking in Azotobacter, as shown by the previous investigation (13). Further characteristics of these types may be obtained from the literature (3).

In these experiments it seemed desirable to study the effect of different amounts of the following fertilizers: (*a*) lime for the purpose of varying the pH of the samples, (*b*) oat straw to increase the organic matter content, (*c*) sodium nitrate in order to determine whether or not small applications of nitrogen would actually stimulate Azotobacter growth whereas larger applications might be expected to depress their activity, and (*d*) triple superphosphate in order to increase the amount of available phosphate.

The lime treatments consisted of pure calcium carbonate. The phosphate used was "Anaconda" triple superphosphate, containing 45.35 per cent available P_2O_5. The oat straw had been ground to pass a 10-mesh screen. The sodium nitrate was of commercial chemical stock.

The different amounts of each of the four fertilizers used for the experiment were as follows:

FERTILIZER	AMOUNTS PER ACRE*			
	0	1	2	3
P = Triple superphosphate.....*pounds*	0	300	...	...
N = Sodium nitrate.....*pounds*	0	100	500	...
O = Oat straw.....*pounds*	0	2,000	...	...
L = Lime (calcium carbonate) x *lime requirement*	0	½	1	3

* Based on 2,000,000 pounds of dry soil.

Combining the different amounts (represented by the subscripts 0, 1, 2, and 3) of the different fertilizers (represented by P for superphosphate, N for sodium nitrate, etc.), in every possible combination according to a factorial design (7), results in the following 48 different groupings representing the treatments used:

Pot number and treatment

1. $P_0N_0O_0L_0$	13. $P_0N_1O_1L_0$	25. $P_1N_0O_0L_0$	37. $P_1N_1O_1L_0$
2. $P_0N_0O_0L_1$	14. $P_0N_1O_1L_1$	26. $P_1N_0O_0L_1$	38. $P_1N_1O_1L_1$
3. $P_0N_0O_0L_2$	15. $P_0N_1O_1L_2$	27. $P_1N_0O_0L_2$	39. $P_1N_1O_1L_2$
4. $P_0N_0O_0L_3$	16. $P_0N_1O_1L_3$	28. $P_1N_0O_0L_3$	40. $P_1N_1O_1L_3$
5. $P_0N_0O_1L_0$	17. $P_0N_2O_0L_0$	29. $P_1N_0O_1L_0$	41. $P_1N_2O_0L_0$
6. $P_0N_0O_1L_1$	18. $P_0N_2O_0L_1$	30. $P_1N_0O_1L_1$	42. $P_1N_2O_0L_1$
7. $P_0N_0O_1L_2$	19. $P_0N_2O_0L_2$	31. $P_1N_0O_1L_2$	43. $P_1N_2O_0L_2$
8. $P_0N_0O_1L_3$	20. $P_0N_2O_0L_3$	32. $P_1N_0O_1L_3$	44. $P_1N_2O_0L_3$
9. $P_0N_1O_0L_0$	21. $P_0N_2O_1L_0$	33. $P_1N_1O_0L_0$	45. $P_1N_2O_1L_0$
10. $P_0N_1O_0L_1$	22. $P_0N_2O_1L_1$	34. $P_1N_1O_0L_1$	46. $P_1N_2O_1L_1$
11. $P_0N_1O_0L_2$	23. $P_0N_2O_1L_2$	35. $P_1N_1O_0L_2$	47. $P_1N_2O_1L_2$
12. $P_0N_1O_0L_3$	24. $P_0N_2O_1L_3$	36. $P_1N_1O_0L_3$	48. $P_1N_2O_1L_3$

In each of 48 glazed quart pots was placed 900 gm. of the air-dried Grundy soil; and in a similar set of pots, 900 gm. of the air-dried Clinton soil. Each portion of each of the two soil types was then given a fertilizer treatment to correspond to one of the 48 treatments listed. The selection and treatments were made at random. Each portion of soil was then brought up to optimum field moisture content and inoculated with 10 cc. of a suspension of Azotobacter. At the end of 3, 6, and 9 months' incubation at room temperature, the soils were sampled for the presence of the bacteria.

The Azotobacter inoculum used in these experiments was obtained by suspending in tap water the growth which could be scraped from the surface of large agar slants of the bacteria. The species used for this purpose consisted of pure cultures of *Az. vinelandii*, *Az. beijerinckii*, and *Az. chroococcum*[3] and of 13 unidentified cultures which had been isolated for the purpose by means of Winogradsky's rapid sodium benzoate method (2) from different Iowa soils. The inoculum consisted of a combination of all of the cultures.

Sterile sampling tubes were used for the collection of the samples from the potted soils for cultural purposes, the samples being collected from several places in the pot to a depth of 3 or 4 inches and placed in small "coin" envelopes.

The presence and the activity of the Azotobacter in these soils were determined by a modification of Curie's agar plate method (6). A nitrogen-free agar medium using mannitol and commercial cane sugar as energy source was prepared, poured into large petri dishes in 50-cc. quantities, and allowed to solidify. The soil to be tested was dried over night, passed through a 20-mesh and then through a 40-mesh screen, and the fraction caused by the 40-mesh screen was used for the determination. Five hundred milligrams of the sieved soil was sprinkled evenly over the surface of duplicate plates of the medium, which were then incubated at 28°C. for 7 days. After this time, the amount of nitrogen fixed by the organisms was determined by the Kjeldahl method according to the recommendations of the Association of Official Agricultural Chemists (1).

The pH of the samples taken was determined electrometrically on a 1–2.5 soil:water ratio by the quinhydrone method as described by Biilmann and Jensen (2). The lime requirement determinations were made according to the method of Hardy and Lewis (10) and by the potassium thiocyanate method (11). The data were analyzed statistically by the analysis of variance method (15).

RESULTS AND DISCUSSION

The average quantities of nitrogen fixed on nitrogen-free agar plates after 3, 6, and 9 months' incubation of the samples of Clinton silt loam are shown in table 1. The pH values of the samples were also determined, but since they differed very little from sample to sample for the same lime-treated soils and

[3] These were obtained through the courtesy of N. R. Smith of the Bureau of Plant Industry.

since the summarized results are given in figure 1, they will not be presented. The results of the analysis of variance of the data are shown in table 2. The unlimed samples were not included in this analysis, since these soils did not

TABLE 1

Average quantities of nitrogen, in milligrams, fixed per gram on nitrogen-free agar plates for variously treated pots of Clinton silt loam inoculated with Azotobacter and allowed to incubate 3, 6, and 9 months before sampling

TREATMENTS*		Length of the incubation period, in months	P0			P1		
			N0	N1	N2	N0	N1	N2
O0	L0	3	0.0	0.0	0.0	0.0	0.0	0.0
		6	0.0	0.0	0.0	0.0	0.0	0.0
		9	0.0	0.0	0.0	0.0	0.0	0.0
	L1	3	10.80	1.76	1.16	6.44	7.88	7.88
		6	13.40	11.60	13.60	10.80	11.20	3.00
		9	6.80	6.00	6.40	6.80	11.20	7.00
	L2	3	12.28	11.70	12.86	13.16	13.72	9.64
		6	18.20	18.20	16.60	16.20	17.40	17.40
		9	15.40	16.40	14.60	14.00	16.20	14.80
	L3	3	12.28	10.80	13.16	11.70	11.40	13.72
		6	19.40	17.80	18.00	18.80	16.80	17.40
		9	17.20	16.00	16.20	16.40	16.00	16.60
O1	L0	3	0.0	0.0	0.0	0.0	0.0	0.0
		6	0.0	0.0	0.0	0.0	0.0	0.0
		9	0.0	0.0	0.0	0.0	0.0	0.0
	L1	3	1.16	10.50	8.48	2.34	7.88	9.06
		6	13.20	6.80	3.20	13.60	14.80	14.80
		9	11.60	7.90	11.00	12.60	12.00	7.40
	L2	3	10.20	10.50	12.28	11.40	12.86	9.96
		6	15.60	17.80	18.20	18.20	18.00	16.80
		9	14.80	13.60	14.20	15.60	14.20	14.80
	L3	3	11.10	11.98	11.98	12.86	11.40	12.28
		6	17.60	17.20	18.80	18.60	17.40	17.80
		9	15.60	15.40	16.50	16.60	17.00	16.80

* Symbolism explained in section "Experimental."

contain the Azotobacter. An advantage of the factorial design is that any treatment can be dropped without disturbing the value of the experiment.

The data show that the lime treatments influenced highly significantly the growth and activity of the Azotobacter. None of the other treatments significantly influenced their growth in the absence of a treatment with lime.

It is of interest, therefore, to look more closely at the influence of the lime treatments upon the Azotobacter. Each lime treatment was replicated 12 times. All of these were averaged for each lime treatment and the variation in results with time have been plotted in figure 1. The pH values corresponding to their respective lime treatments were also averaged and these data, too, appear in figure 1.

The results show that the original soil with a pH of about 6.17 was unable to support a growth of the bacteria. The remaining lime treatments brought about an environment that was increasingly favorable for the bacteria as the

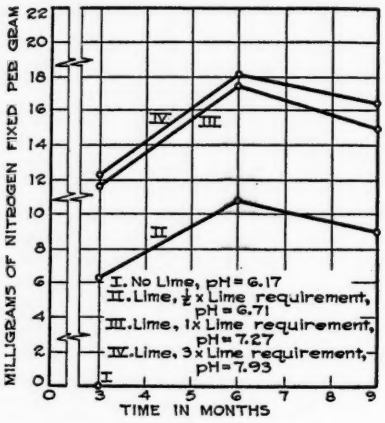
MILLIGRAMS OF NITROGEN FIXED PER GRAM
TIME IN MONTHS
I. No Lime, pH = 6.17
II. Lime, ½ x Lime requirement, pH = 6.71
III. Lime, 1x Lime requirement, pH = 7.27
IV. Lime, 3x Lime requirement, pH = 7.93

FIG. 1

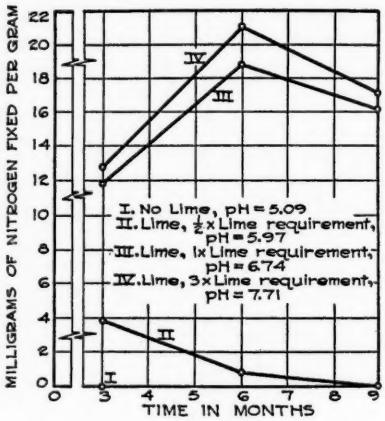
MILLIGRAMS OF NITROGEN FIXED PER GRAM
TIME IN MONTHS
I. No Lime, pH = 5.09
II. Lime, ½ x Lime requirement, pH = 5.97
III. Lime, 1x Lime requirement, pH = 6.74
IV. Lime, 3x Lime requirement, pH = 7.71

FIG. 2

FIG. 1. AVERAGE QUANTITIES OF NITROGEN FIXED ON NITROGEN-FREE AGAR PLATES FOR CLINTON SILT LOAM TREATED WITH LIME, INOCULATED WITH AZOTOBACTER, AND ALLOWED TO INCUBATE 3, 6, AND 9 MONTHS BEFORE SAMPLING

FIG. 2. AVERAGE QUANTITIES OF NITROGEN FIXED ON NITROGEN-FREE AGAR PLATES FOR GRUNDY SILT LOAM TREATED WITH LIME, INOCULATED WITH AZOTOBACTER, AND ALLOWED TO INCUBATE 3, 6, AND 9 MONTHS BEFORE SAMPLING

lime treatment increased. The best growth was obtained when sufficient lime was added to raise the pH of the samples to about the neutral point or above.

An examination of table 2 shows that the oat-straw treatment in the presence of lime influenced the growth and activity of the bacteria. At the end of 9 months' incubation, the "oat-straw x lime" interaction was highly significant. The data show that the influence of the oat straw upon the Azotobacter was more pronounced in the soils treated with the smaller amounts of lime. The effect was not observed when the soil reaction was most favorable for the growth of the bacteria.

Table 2 shows also, that in one instance (at the end of 6 months' incubation) the "phosphate x oat-straw" interaction was significant. In this case, available phosphorus seemed to stimulate the growth of the bacteria in the presence

of oat straw but not in its absence. Perhaps the phosphorus stimulated the growth of other soil microörganisms in their decomposition of the oat straw, thus either releasing decomposition products of the oat straw which could be utilized by the Azotobacter as sources of energy, or widening the C:N ratio of the soil by the assimilation of the available nitrogen present so that the Azotobacter, which fix nitrogen from the atmosphere, could successfully compete for the nutrients available and make a better growth.

TABLE 2

Analysis of variance of the milligrams of nitrogen fixed by the Azotobacter colonies from Clinton silt loam sampled after 3, 6, and 9 months' incubation

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE		
		Incubation period		
		3 months	6 months	9 months
Between treatments:*				
P	1	3.10	0.41	2.95
N	2	1.24	6.75	1.13
O	1	0.48	0.19	3.80
L	2	125.98†	188.25†	187.95†
Interactions:				
P x N	2	1.33	1.52	3.97
P x O	1	1.33	43.12‡	0.51
P x L	2	1.08	1.69	1.00
N x O	2	16.54	0.49	2.98
N x L	4	2.74	6.33	1.02
O x L	2	1.96	0.27	13.03†
Remainder	16	7.23	7.53	1.74
Total	35	....	....	....

* Symbolism explained in section "Experimental."
† Highly significant.
‡ Significant.

None of the individual treatments other than lime were essential for the growth of the bacteria in Clinton silt loam. In fact, other than oat straw and perhaps available phosphorus, none of the other treatments, regardless of their combination, seemed to exert any influence upon the bacteria.

The average milligrams of nitrogen fixed per gram on nitrogen-free agar plates after 3, 6, and 9 months' incubation of the samples of Grundy silt loam are shown in table 3. The analyses of variance of the data are given in tables 4 and 5. The unlimed samples were not included in the analysis in table 4, since these soils did not contain the bacteria. In addition, in table 5, the samples treated with lime equivalent to one half the lime requirement of

Grundy silt loam were not included, since these samples, too, at this sampling period did not contain the bacteria.

As in the samples of Clinton silt loam, the analyses of variance (tables 4 and 5) show that the lime treatments influenced highly significantly the growth

TABLE 3

Average quantities of nitrogen, in milligrams, fixed per gram on nitrogen-free agar plates for variously treated pots of Grundy silt loam inoculated with Azotobacter and allowed to incubate 3, 6, and 9 months before sampling

TREATMENTS*		Length of incubation period, in months	P_0			P_1		
			N_0	N_1	N_2	N_0	N_1	N_2
O_0	L_0	3	0.0	0.0	0.0	0.0	0.0	0.0
		6	0.0	0.0	0.0	0.0	0.0	0.0
		9	0.0	0.0	0.0	0.0	0.0	0.0
	L_1	3	1.46	2.92	4.68	4.38	1.76	2.98
		6	0.00	1.20	0.80	0.40	0.00	0.80
		9	0.00	0.00	0.00	0.00	0.00	0.00
	L_2	3	10.50	12.28	12.86	13.44	11.98	10.50
		6	17.80	19.60	19.20	19.40	20.00	17.80
		9	16.20	16.60	15.00	16.20	15.60	16.20
	L_3	3	13.72	12.86	13.16	11.70	13.76	11.70
		6	20.80	21.60	21.80	22.40	22.80	19.80
		9	16.80	17.00	18.60	18.20	16.80	16.80
O_1	L_0	3	0.0	0.0	0.0	0.0	0.0	0.0
		6	0.0	0.0	0.0	0.0	0.0	0.0
		9	0.0	0.0	0.0	0.0	0.0	0.0
	L_1	3	8.76	9.04	2.08	2.34	2.08	4.68
		6	1.00	1.40	3.60	0.20	1.40	1.00
		9	0.00	0.00	0.00	0.00	0.00	0.00
	L_2	3	11.98	11.70	11.98	13.16	12.86	10.05
		6	17.00	18.80	17.80	19.80	18.20	19.00
		9	16.20	16.20	15.70	17.20	16.20	16.60
	L_3	3	13.44	13.16	10.80	13.44	11.70	11.40
		6	20.60	19.60	21.20	20.20	20.40	20.80
		9	16.60	16.00	17.00	17.40	17.60	17.80

* Symbolism explained in section "Experimental."

and activity of the Azotobacter. No other treatment or combination of treatments significantly affected the bacteria, with the exception of the oat-straw treatment in the absence of large amounts of lime at the 6 months' sampling period. The results for each of the 12 samples which had received the same lime treatment, regardless of other materials added, for each sampling

TABLE 4

Analysis of variance of the milligrams of nitrogen fixed by the Azotobacter from Grundy silt loam sampled after 3 and after 6 months' incubation

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE	
		Incubation period	
		3 months	6 months
Between treatments:*			
P	1	4.709	0.010
N	2	2.819	0.654
O	1	1.988	0.490
L	2	279.550†	1,439.674†
Interactions:			
P x N	2	0.877	2.263
P x O	1	2.026	0.010
P x L	2	2.981	1.423
N x O	2	3.474	1.563
N x L	4	0.187	0.659
O x L	2	4.247	3.103‡
Remainder	16	3.247	0.627
Total	35	.....	.....

* Symbolism explained in section "Experimental."

† Highly significant.

‡ Significant.

TABLE 5

Analysis of variance of the milligrams of nitrogen fixed by the Azotobacter from Grundy silt loam sampled after 9 months' incubation

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE
Between treatments:*		
P	1	0.882
N	2	0.252
O	1	0.015
L	1	6.615†
Interactions:		
P x N	2	0.272
P x O	1	1.215
P x L	1	0.015
N x O	2	0.015
N x L	2	0.545
O x L	1	0.735
Remainder	9	0.454
Total	23	.....

* Symbolism explained in section "Experimental."

† Highly significant.

period were averaged, therefore, and have been plotted in figure 2. The pH values corresponding to the respective lime treatments were also averaged, and these data also appear in figure 2.

The results show that the original soil with a pH of about 5.09 was unable to support a growth of the bacteria. The samples which had been treated with lime equivalent to one half the lime requirement were also unable to support a growth of the bacteria. The average pH of these samples was about 5.97, which is in accord with the limiting value for the growth of the bacteria in mineral field soils, i.e., pH 6.0, as postulated by other investigators (8, 9). It should be noted, however, that the Azotobacter remained in these soils for rather a long time, still being detected after 6 months' incubation. This is in contrast to the result obtained with Clinton silt loam, which had a higher initial pH of 6.17 but in which the organisms died off completely in 3 months.

The remaining lime treatments brought about an environment that was increasingly favorable for the growth of the Azotobacter as the lime treatment increased. An addition of lime equivalent to three times the lime requirement and sufficient to raise the pH to about 7.71 had very little effect, however, in improving the environment for the growth of the Azotobacter over that of the soils which had received lime equivalent to the lime requirement and sufficient to raise the pH of the samples to about 6.74.

Here again the contrast between Grundy and Clinton silt loams is very marked. It may be observed by a comparison of figures 1 and 2, that at a much lower pH, Grundy silt loam was able to support an Azotobacter flora which fixed more nitrogen than did the flora of Clinton silt loam. At the same pH, therefore, Grundy silt loam was able to furnish an environment which was more favorable to the growth of the Azotobacter than was that of Clinton silt loam. One of the reasons for this probably rests with the higher organic matter content of Grundy silt loam. A previous investigation of the factors which influenced the presence of the Azotobacter in Iowa soils (13) showed that when other factors were favorable, the amount of growth which the Azotobacter would make in a soil was most closely associated with the organic matter content.

Phosphorus or nitrogen differences between the two types were probably not of any great significance, since these treatments, with the possible exception of phosphorus in the presence of an oat-straw treatment for Clinton silt loam, did not influence the growth and activity of the bacteria in either soil, at least not to a significant extent as determined by the methods used in this investigation. This does not support one conclusion of the correlation studies (13) to the effect that the available phosphate content of Iowa soils may be of importance in limiting the growth of the bacteria in them.

This finding is interesting in view of the fact that crops on all of these soils respond favorably to applications of phosphate fertilizers. Vandecaveye and Anderson (16) also found that phosphorus failed to stimulate Azotobacter growth. Both observations support the results obtained by Burk and Line-

weaver (4) with pure cultures of the bacteria to the effect that phosphorus is not essential for the growth of the bacteria except in extremely small amounts.

The findings of Winters (22) to the effect that applications of sodium nitrate, at the rate of 100 pounds an acre, to some New York soils stimulated the growth of the Azotobacter whereas all larger applications depressed it, was not supported by these results either. Neither was the work of Winogradsky (19, 20) or of Ziemiecka (24) supported by these results. Both these workers found that the application of fixed nitrogen to the soil in large amounts depressed the Azotobacter flora and in many cases entirely eliminated them from the soil. They, however, sampled plots of soil which had received repeated dressings of nitrogenous fertilizers over a period of years and to which, consequently, a large total amount of nitrogen had been added. Winogradsky (21) makes a statement somewhat to the same effect, as follows: "Regular and abundant dressings [of nitrogenous fertilizers] would tend to reduce its [Azotobacter] density or even make the species disappear altogether."

SUMMARY AND CONCLUSIONS

A study was made of the influence of different fertilizers or combinations of fertilizers upon the growth and activity of Azotobacter in typical samples of Clinton and Grundy silt loams. These soils were chosen for the experiment because they represented a wide variety of soil conditions and, in addition, had been found to be generally lacking in Azotobacter (13).

The fertilizers used in these experiments were (*a*) lime to vary the pH of the samples, (*b*) oatstraw to increase the organic matter content, (*c*) sodium nitrate to determine whether or not small amounts of nitrogen added to these soils would actually stimulate Azotobacter growth, and (*d*) triple superphosphate to increase the amount of readily available phosphate.

The results showed that an addition of lime to these soils was essential for the prolonged growth of the bacteria and that an amount sufficient to raise the pH to near neutrality was all that was necessary to improve the environment so that the bacteria would remain active for at least 9 months.

None of the individual treatments other than lime were essential for the growth of the bacteria in these soils. Indeed, except for oat straw, none of the treatments other than lime, regardless of their combination, seemed to exert any influence upon the bacteria.

There was some evidence when lime was present, however, that other treatments may aid the growth of the bacteria. This was particularly true for the oat-straw treatment of Clinton silt loam.

It was found that at the same pH, Grundy silt loam presented a better medium for the growth and activity of the Azotobacter than did Clinton silt loam. One of the reasons for this difference was attributed to the higher organic matter content of Grundy silt loam.

The limiting pH value for the growth of the Azotobacter in these soils was shown to approach a pH of 6.0, but it was pointed out that the actual limiting value for the different types undoubtedly varied with the general soil conditions.

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EFFECT OF ORGANIC MATERIALS AND FERTILIZER TREATMENTS UPON THE SOLUBLE NUTRIENTS IN SOILS[1]

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Soil organic matter is the seat of biological life and processes important to soil fertility. Oxidations and decompositions brought about by microörganisms result in nitrates, sulfates, phosphates, and bicarbonates that are themselves important plant nutrients or active solvents of mineral matter of soils. This study undertakes to investigate the significance of organic decay and the activity of microörganisms in relation to the solubility of nutrients in soils.

METHODS OF STUDY

For the data of tables 1 and 2 grass clippings, alfalfa hay, and wheat straw were dried and ground. Water extracts of these materials before and after their addition to the soil were used for analysis.

The data of tables 3 and 4 represent results from soils treated in the greenhouse. Two soils were used, one from the western Oregon hills, acid and leached (Aiken clay loam), the other from southern Oregon, about neutral in reaction and well supplied with bases (Meyer clay adobe).

The data reported in tables 5 and 6 were obtained from soils incubated in the laboratory in tumblers. The treatments were made at rates shown in the tables, and the soils were kept moist during the incubation periods. Urea was used as a source of nitrogen for the nitrification study. Starch and dextrose were used as energy materials for microörganisms to stimulate vigorous activity and evolution of carbon dioxide.

Water extracts at the stated periods were analyzed by standard methods. The phenol-disulfonic acid method was used for nitrates. Calcium was precipitated as oxalate and titrated with permanganate. Potassium was precipitated as cobaltinitrite and titrated with permanganate (5).

Table 7 provides data from a soil profile sampled as shown. Humus was determined by the Walkley and Armstrong method (14). Available phosphorus was determined by the Truog method (13). Exchangeable calcium and potassium were obtained by extraction with 0.05 *N* HCl. Carbonates and bicarbonates were determined by double titration with phenolphthalein and methyl orange as indicators.

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The soil for which profile data are given supported a bearing orchard of Persian walnuts.

RESULTS OF STUDY

Such organic materials as grass, weeds, straw, and legumes commonly returned to the soil, contain a considerable portion of soluble nutrients important to plant growth. Some of these nutrients are water soluble before decay occurs. Other nutrients are liberated as decomposition proceeds. Absorption processes in the soil, microörganisms, and plants each remove a portion

TABLE 1

Fixation of soluble potassium of grass, alfalfa, and straw by Chehalis silt loam

MATERIAL	WATER-SOLUBLE K IN MATERIAL	K ADDED TO SOIL	K OBTAINED IN WATER EXTRACT	K INCREASE FROM PLANT MATERIALS	K FIXED BY SOIL	FIXATION
	per cent	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*per cent*
Grass	2.49	249.5	87.4	58.0	191.5	76.7
Alfalfa	1.24	124.5	52.0	22.6	101.9	81.8
Straw	1.48	148.0	55.8	26.4	121.6	82.2
Soil	....	.....	29.4	....	.....	....

TABLE 2

Fixation of soluble calcium of grass, alfalfa, and straw by Aiken clay loam

MATERIAL	WATER-SOLUBLE Ca IN MATERIAL	Ca ADDED TO SOIL	Ca OBTAINED IN WATER EXTRACT	Ca INCREASE FROM PLANT MATERIALS	Ca FIXED BY SOIL	FIXATION
	per cent	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*per cent*
Grass	0.28	28	24	20	8	28.6
Alfalfa	0.59	59	18	14	45	76.3
Straw	0.28	28	14	10	18	64.3
Soil	....	..	4	..	..	....

of the solutes from solution. The data of tables 1 and 2 indicate soil absorption processes in relation to soluble calcium and potassium.

Column 2, represents the percentage, dry basis, of water-soluble potassium and calcium found in dried and ground grass, alfalfa, and straw. Column 3 shows the result of adding 1 per cent of the dry materials to the respective soils, or the amount of soluble potassium or calcium provided from the plant materials in the soil. One per cent of green grass adds enough potassium to make 249.5 p.p.m. in the soil and enough soluble calcium to make 28 p.p.m. Column 4 shows how much soluble potassium or calcium could be extracted by shaking the mixture of 1 per cent dry materials and of soil with water for 1 hour and filtering. With the grass only 87.4 p.p.m. of potassium and 24 p.p.m. of calcium were extracted. The soils supplied 29.4 p.p.m. of potassium and

4 p.p.m. of calcium respectively with no plant materials added. The grass, therefore, contributed only 58 (column 5) out of its possible 249.5 p.p.m. of potassium and 20 out of its possible 28 p.p.m. of calcium, or 191.5 p.p.m. of potassium (column 6) and 8 p.p.m. of calcium supplied by the grass are fixed by the soil. These quantities are equivalent to 76.7 and 28.6 per cent fixation respectively. Similar data are provided for the other materials.

TABLE 3

Water-soluble potassium in potted soils incubated in the greenhouse

TREATMENT*	CHECK	ALFALFA 5 TONS	MANURE 5 TONS	STRAW 5 TONS	COMPLETE 2,200 POUNDS	MANURE 2½ TONS + COMPLETE 1,100 POUNDS	SUPERPHOSPHATE 600 POUNDS	MANURE 2½ TONS + SUPERPHOSPHATE 300 POUNDS
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Meyer clay adobe								
Incubation period:								
2 months	21	31	32	22	31	31	20	24
6 months	19	26	27	17	26	22	17	20
12 months	18	27	26	19	23	25	16	14
Average	19	28	28	19	27	26	18	19
Increase from treatment	..	9	9	0	8	7	−1	0
Aiken clay loam								
Incubation period:								
2 months	11	23	26	12	20	24	9	14
6 months	11	23	33	16	27	32	14	27
12 months	10	26	31	16	23	33	9	27
Average	11	24	30	15	23	30	11	23
Increase from treatment	..	13	19	4	12	19	0	12

* The complete was made by mixing six parts sodium nitrate, three parts superphosphate, and two parts potassium sulfate. Rates are for 2,000,000 pounds of soil.

The data in tables 3 and 4 represent the combined effect of the soil and microorganisms. The net effect of adding fertilizers or organic materials depends upon the nature of the materials and of the soils and upon the period of time that has elapsed after incorporation of the materials with the soil.

There is consistently more soluble calcium in the Meyer soil than in the Aiken, both before and after treatments. The effect of the treatments in increasing soluble calcium is not consistently greater in either soil. Alfalfa, manure, and complete fertilizer have had the most pronounced effect upon

TABLE 4

Water-soluble calcium in potted soils incubated in the greenhouse

TREATMENT*	CHECK	ALFALFA 5 TONS	MANURE 5 TONS	STRAW 5 TONS	COMPLETE 2,200 POUNDS	MANURE 2½ TONS + COMPLETE 1,100 POUNDS	SUPERPHOSPHATE 600 POUNDS	MANURE 2½ TONS + SUPERPHOSPHATE 300 POUNDS
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Meyer clay adobe								
Incubation period:								
2 months	52	100	72	32	116	76	50	60
6 months	60	84	60	48	92	56	60	40
12 months	44	68	49	42	64	54	49	43
Average	52	84	60	41	91	62	53	48
Increase from treatment	..	32	8	−11	39	10	1	−4
Aiken clay loam								
Incubation period:								
2 months	24	40	32	24	40	32	22	24
6 months	20	36	24	20	40	28	24	24
12 months	16	46	25	26	38	28	23	26
Average	20	41	27	23	39	29	23	25
Increase from treatment	..	21	7	3	19	9	3	5

* See note table 3.

TABLE 5

Water-soluble nitrate, calcium, and potassium in Aiken clay loam

TREATMENT	AFTER 2 WEEKS			AFTER 4 WEEKS			AFTER 6 WEEKS		
	NO_3 as N	Ca	K	NO_3 as N	Ca	K	NO_3 as N	Ca	K
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1. Check	40	35	6	40	45	9	43	52	21
2. Lime 2 tons	40	60	6	40	70	9	50	90	21
3. Urea 200 p.p.m. N	60	15	6	67	35	11	133	95	30
4. Lime and urea	75	40	18	100	100	18	218	230	40
5. HNO_3 = N of urea	240	180	20	214	190	17	250	200	63
6. HNO_3 + lime	255	270	24	250	275	20	250	285	54
Average of 1, 2, 3	47	37	6	49	50	10	75	79	24
Average of 4, 5, 6	190	163	21	188	188	18	239	238	52

both soils, in part, no doubt, because more potential solutes are added in these materials.

With respect to potassium, the effects are rather consistently greater on the Aiken soil, which is lower in soluble potassium, than are the effects on the Meyer soil. The treatments which increased soluble calcium most also increased soluble potassium to the greatest extent, perhaps for the same reason.

Straw fails to show much effect in increasing soluble calcium or potassium, probably partly because of the depressing effect upon nitrate, which serves

TABLE 6

Water-soluble bicarbonate, calcium and potassium in Meyer clay adobe

TREATMENT	AFTER 3 DAYS			AFTER 10 DAYS			AFTER 21 DAYS		
	HCO_3 as Ca	Ca	K	HCO_3 as Ca	Ca	K	HCO_3 as Ca	Ca	K
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Check	53	72	39	33	92	33	1	118	31
Starch 1 per cent	73	56	32	82	40	23	10	32	17
Lime 5 tons	212	138	44	171	164	32	44	164	31
Lime 5 tons, starch 1 per cent	268	136	30	211	92	26	52	68	17
Sugar 1 per cent	122	98	23	82	40	21	8	42	17
Sugar 1 per cent, lime 5 tons	544	200	27	268	104	25	65	75	21

TABLE 7

Soluble nutrients in an 8-foot profile, Olympic clay loam

DEPTH	ORGANIC MATTER	AVAILABLE PHOSPHORUS	WATER-SOLUBLE POTASSIUM	EXCHANGEABLE POTASSIUM	WATER-SOLUBLE CALCIUM	EXCHANGEABLE CALCIUM
inches	*per cent*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
0–6	2.58	28.4	20.3	232.7	65.9	1,166.1
6–12	1.86	20.1	10.9	168.1	28.4	1,269.6
12–24	0.83	7.5	8.7	144.3	20.1	1,024.9
24–36	0.63	10.7	7.2	142.8	8.0	935.0
36–48	0.49	14.0	1.1	112.9	15.7	1,061.3
48–60	0.26	14.0	....	88.0	4.0	1,111.0
60–72	0.14	14.0	....	79.0	3.4	932.6
72–84	0.13	14.0	....	105.0	3.0	949.0
84–96	0.12	14.0	....	61.0	2.9	1,045.1

as a carrier of cations (11). Soluble complete fertilizer in a rather liberal quantity has no greater effect than have the higher nitrogen organics, manure and alfalfa. In all cases, soluble nutrients are undoubtedly fixed by both the soil and the microörganisms (11).

Superphosphate, which supplies soluble calcium, has had little effect upon either soluble calcium or potassium in the soil, probably because of fixation of the calcium. No direct effect upon potassium can be expected, and little indirect effect is apparent.

Several acids originate from organic decay. Of the strong acids so formed, nitric, as nitrates, is one of the most abundant. The solvent effect of nitric acid has been brought out by a number of workers (1, 3, 7, 10, 12). Carbonic is an abundant weak acid resulting from decay. A legume with 2 per cent nitrogen and 40 per cent carbon is capable of liberating through decay many times as much carbonic as nitric acid. The data of tables 5 and 6 indicate, however, that more solvent effect is due to nitric than to carbonic acid, particularly in soils that are acid in reaction. The limitations of carbonic acid under field conditions as a solvent affecting plant nutrition have been brought out by others (2, 8, 9).

The importance of the solvent effect of nitrification is indicated in table 5 where urea, under conditions favorable to nitrification, was added to the soil. The soil minerals and limestone furnish the bases necessary for the nitrification process. Although calcium and potassium are important bases in the nitrification process, others may also serve this function. The urea is never nitrified sufficiently to produce a solvent effect upon the calcium and potassium of the soil equal to that of an equivalent amount of nitric acid added direct.

The 200 p.p.m. of nitrogen, if all nitrified, is capable of combining with approximately 285 p.p.m. of calcium or with 560 p.p.m. of potassium in the soil. In most cases, therefore, considerable other matter must have been brought into solution as nitrate. The largest amounts of calcium and potassium are found in solution at the time when nitrates are present in largest amounts.

The meager solvent effect of carbonic acid, likewise on calcium and potassium only, is shown in table 6. The bicarbonate found was converted to its calcium equivalent for convenience of comparison. Only a little bicarbonate could be detected in the water extract of the untreated soil. Treatment of the soil with starch or sugar alone, resulted in some extractable bicarbonate at first. The effect nearly disappeared in a short time, however. When starch or sugar was used with calcium carbonate on the soil, a considerable quantity of extractable bicarbonate was produced. The effect lasted through a 10-day period. Calcium carbonate alone resulted in the largest amount of water-soluble calcium after the first 3-day flush production of carbon dioxide. The data indicate that carbon dioxide evolution, stimulated by feeding the microörganisms easily available energy material, has little measurable effect in dissolving calcium or potassium from the minerals of the soil. In fact, the microörganisms may utilize and remove from solution some of the soluble potassium already present (11). The data of tables 5 and 6 indicate that minerals of the soil are brought into solution more by the nitrification process than through the solvent effect of carbonic acid (12).

The effect of organic materials and of fertilizers added principally to the topsoil should be reflected in the distribution of soluble nutrients in the soil profile. This effect is shown in table 7 for an 8-foot profile of Olympic clay loam. More water-soluble calcium and potassium, exchangeable potassium,

and available phosphorus occur in the top 6 inches than at any other depth. The negligible amount of water-soluble potassium and calcium below 4 feet must be due in part to lack of the solvents that are normally produced from humus in the topsoil.

The comparatively large amount of exchangeable potassium in the topsoil is probably due to the return of organic residues. Most of the potash removed by plant growth remains in the stalk and foliage of plants and naturally returns to the soil with these materials. The humus of the topsoil undoubtedly supplies a considerable amount of active organic colloidal complex for absorbing and holding the potash in an available or exchangeable form.

The amount of exchangeable calcium is much larger than the amount of exchangeable potassium and appears to be less influenced by organic matter or surface additions. Available phosphorus (13) is relatively low in this soil, though it is highest in the surface. The highest concentration of soluble nutrients prevails in the surface soil in spite of crop removal, which is likewise greatest in the surface soil. The distribution of humus and of water-soluble potassium and calcium presented here is typical of 30 soils that have been studied but are not reported because of close similarity.

Greenhouse studies on the soil profile by 1-foot horizons showed that the soil below 4 or 5 feet became nearly sterile insofar as plant growth was concerned. Sunflowers grown as indicator plants germinated, produced two seed leaves, and then died. The subsoils of the more producive soils were noticeably better in this respect than were the subsoils of unproductive types.

DISCUSSION OF RESULTS

When moisture and temperature conditions are favorable, microörganisms are stimulated to activity by the addition of fresh organic materials to the soil. In a short time evolution of carbon dioxide becomes profuse, and the maximum dissolving effect of carbonic acid is evident. Easily soluble material such as free limestone in the soil is brought into solution. The more difficultly soluble minerals such as the natural potash minerals of the soil are not attacked sufficiently to affect the soluble potash significantly. Neither are the exchangeable forms of calcium and potassium in the soil attacked to an appreciable extent by carbonic acid. This is not assumed to indicate what the plant may be able to do with carbon dioxide excreted from the root system.

Work by others (8, 9) indicates that the importance of carbon dioxide as a solvent affecting plant nutrition may have been overemphasized. Apparently it is easier to demonstrate solution by an artificially prepared carbonic acid (4) than to demonstrate that such a reaction liberates plant nutrients under field conditions. There is, however, no doubt of the importance of carbon dioxide production in soils (2, 7) and perhaps little doubt that carbonic acid has its greatest solvent action, and probably its greatest significance to plant nutrition, in neutral or alkaline soils (2, 4, 12).

High-protein materials yield nitrogen as ammonia, which is oxidized to

nitrous acid. This acid combines with a base, and oxidation to the nitrate follows. Nitric acid in the soil, in the form of its salts, is an important carrier of nutrients necessary for plant growth. The action of the strong acids, of which nitric is typical, is much more pronounced in attacking soil minerals than is the action of weak carbonic acid, though carbonic acid is produced more abundantly than any of the stronger acids. This is increasingly significant as soils become more acid (12).

The common plant residues, manure, and fertilizers applied to soils furnish soluble nutrients, a large part of which are fixed by the soil and held until liberated by some solvent produced by natural soil processes. Nitrification is a most important process serving the function of liberating not only nitrogen, but the important nutrient bases (3, 12). Vigorous nitrification may not only supply nutrients to plants but may cause loss of nutrients by leaching, as is indicated by drainage studies (6). Likewise, carbonic acid is perhaps responsible for some leaching losses, especially the loss of calcium after a liberal application of limestone.

An important function of humus and clay in the soil, therefore, is to hold plant nutrients for gradual liberation by biological processes.

Soil building necessitates a system of management which will provide for adequate and regular humus renewal, to build up biological activity to a point where nutrient liberation is adequate for crop needs. Fertilizers should supply those nutrients which biological activities cannot be made to liberate. Fertilization may serve, in part, to stimulate greater biological action and, in part, to supply supplemental nutrients.

The study of the behavior of the soil profile indicates that active humus, microörganisms, soluble nutrients, and plant growth are closely correlated. The top 6 to 12 inches is the best source of nutrients. Below the zone of active humus and vigorous biological processes, the soil horizons become less productive of soluble nutrient materials. In the greenhouse, many raw soil materials below 4 or 5 feet are incapable of supplying much soluble nutrient or of supporting much plant growth.

The rate of humus renewal, rather than the humus level, probably needs more emphasis in the management of soils. A few thousand pounds of fresh material, rich in nitrogen and minerals, may have far greater significance in the liberation of plant nutrients and the production of crops than many tons of the old inert humus of leached and depleted soils. Legumes, residues, and green materials of any sort serve admirably for these functions.

SUMMARY

Organic materials commonly returned to the soil contain a considerable quantity of water-soluble nutrients, the greater portion of which may be absorbed by the soil colloids, organic and inorganic, and held against future crop needs.

In a similar manner soluble fertilizers when added to soils are partially

absorbed and held. Microörganisms no doubt play an important part in the absorption of both nitrogen and the inorganic nutrients.

The liberation of nutrients depends largely upon biological processes and especially upon the solvent effect of the strong acids such as nitric acid. The concentration of cation solutes tends, in general, to follow the abundance of nitrates in the soils studied.

The weak carbonic acid does not appear to cause much solvent action, particularly in acid soils; in neutral or alkaline soils the effect of carbonic acid is probably of greater significance.

The greatest quantity of water-soluble nutrients is found in the surface soil, where organic matter is most abundant and where biological processes are most active.

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REACTION BETWEEN AMMONIA AND SOILS

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Ammonia occupies a unique position among alkalies, as it is volatile and can thus be displaced by almost any other base on heating. The so-called "adsorption" of ammonia by soils has been used as a measure of the amount of colloids in soils (2, 3). We have already shown that the titration curve of soil acidoid with ammonia is no different from similar titration curves with other bases (4). The amount of ammonia that can react with a soil is therefore a function of the pH value: the lower the pH value, the larger the quantity of ammonia retained by it. A knowledge of the exact relationship is not only of theoretical interest but of practical value, as nitrogen in the form of ammonia plays an important part in plant nutrition. The transformation of ammonia into nitrites and nitrates must be governed by the ability of the soil to retain any ammonia produced in the first instance. It is obvious that at very high pH values any ammonia produced will be lost at once, and deficiency of nitrogen would thus be an important factor in causing infertility of alkali soils.

Four soils were used for this study, a black cotton soil (P.C. 13), a typical Punjab soil (P.C. 61), a lateritic soil (P.C. 6), and a clay alluvium (P.C. 123). Exchangeable bases were removed from these soils by treatment with 0.05 *N* HCl as usual. The titration curves of these soils with NaOH and ammonia are given in figures 1–4. With soil P.C. 13, increasing amounts of Na, Ca, Mg, Sr, and Li were used to obtain different pH values; with the other three soils, only Na and Ca were used.

In order to establish the relation between ammonia reaction and pH value of an ordinary buffer solution, the universal buffer mixture of Predaux and Ward (1) brought to different pH values with NaOH and $Mg(OH)_2$ was used. The procedure in every case was to add excess of ammonia to the buffer or soil suspension, boil to half the volume, and then after adding excess of lime determine by distillation the ammonia retained. Preliminary experiments had shown that when a solution of ammonia is boiled until the volume is reduced to half, all of the free ammonia is driven off.

The results with the universal buffer mixture are given in figure 5, along with the ordinary curve of the buffer mixture with NaOH, $Mg(OH)_2$, and ammonia. It will be seen that the ammonia which reacted with the buffer solution is a function of the pH value and, in fact, denotes the residual portion of the titration curve when a part of the solution has been neutralized with

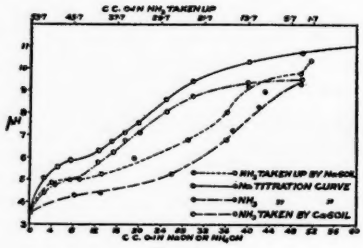

FIG. 1

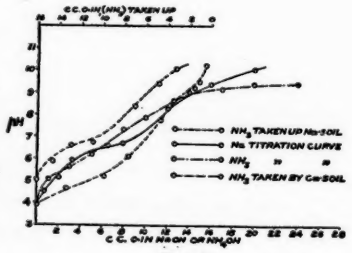

FIG. 2

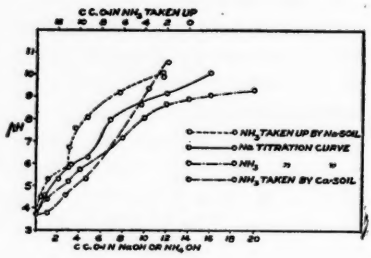

FIG. 3

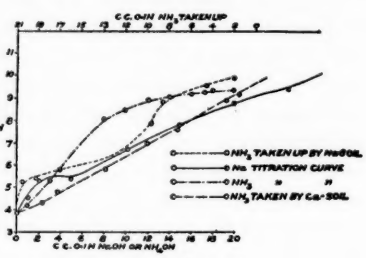

FIG. 4

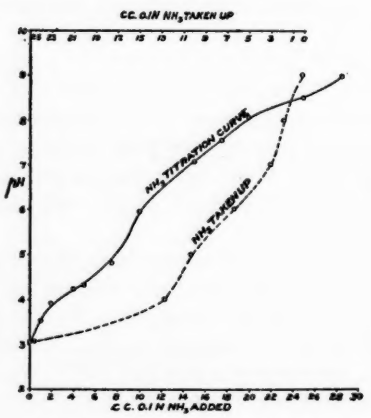

FIG. 5

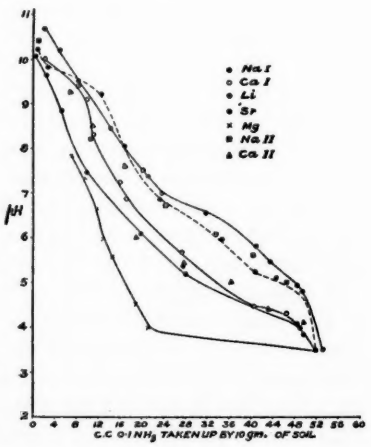

FIG. 6

FIG. 1. TITRATION CURVES AND AMMONIA TAKEN UP BY SOIL P.C. 13

FIG. 2. TITRATION CURVES AND AMMONIA TAKEN UP BY SOIL P.C. 61

FIG. 3. TITRATION CURVES AND AMMONIA TAKEN UP BY SOIL P.C. 6

FIG. 4. TITRATION CURVES AND AMMONIA TAKEN UP BY SOIL P.C. 123

FIG. 5. TITRATION CURVE OF B.D.H. BUFFER WITH AMMONIA

FIG. 6. AMMONIA TAKEN UP BY Na-, Li-, Sr-, Ca-, AND Mg-SOILS (P.C. 13)

NaOH and MgO. These residual titration curves are no different from the straight-forward curves. It is also seen that no ammonia is taken up above pH 9 by the buffer solution.

The four soils were gradually neutralized with different bases, as previously mentioned. The pH values were determined after 48 hours' shaking (4). Then 10 cc. of *N* NH_4OH was added to each suspension, and after 48 hours the suspension was boiled until the volume was reduced to one half. Ammonia was then determined by distillation in the usual way after the addition of excess of lime. The results plotted in figure 6 show the characteristic shape of the residual titration curve. Straight-forward curves in every case are also given for comparison.

The experiment was repeated for Na and Ca ions by allowing the acidoid to take up ammonia, adding and boiling the excess, and then adding increasing amounts of NaOH and $Ca(OH)_2$, followed by boiling. These results, also

TABLE 1

Replacement of ammonia by various hydroxides

0.1 *N* HYDROXIDES ADDED	0.1 *N* NH_3 DISPLACED			
	NaOH added to soil		$Ca(OH)_2$ added to soil	MgO added to soil
	Before boiling	After boiling	After boiling	After boiling
cc.	*cc.*	*cc.*	*cc.*	*cc.*
2.5	2.2	3.2	3.7	....
5.0	5.3	4.9	4.9	4.3
10.0	8.8	8.8	8.4	5.6
15.0	13.8	13.3	12.8	9.3
20.0	15.2	15.3	17.0	10.0
25.0	20.6	18.8	18.3	14.3
30.0	21.7	20.8	21.3	14.6
40.0	25.3	23.1	23.3	16.4

plotted in figure 6, show that the final equilibrium is the same whether ammonia is added after the addition of NaOH or $Ca(OH)_2$, or before. It is also seen that a certain amount of $Ca(OH)_2$ and NaOH added to ammonia soil does not drive out an equivalent amount of ammonia. The results given in table 1 show that the relation between the ammonia taken up and the amount of exchangeable base in the soil is valid only when the pH value, not the amount of alkali added, is taken into consideration.

Further experimental evidence to rule out the possibility of adsorption of ammonia by soil seems superfluous. The almost perfect relationship between pH and ammonia retention, both by buffer solution and soils, is proof positive that both are governed by the same laws of chemical reaction. In order to convince ourselves that adsorption plays no part whatsoever, an attempt was made to study the retention of ammonia by well-known adsorbents such as charcoal and freshly precipitated $BaSO_4$. The latter retained absolutely no

ammonia, and the former, only 1 m.e. per 100 gm., a quantity so small that slight acidic impurity in the charcoal would completely account for it. A study was also made of the reaction of NH_3 with silicic acid, which was prepared from sodium silicate by the addition of HCl followed by filtration and leaching until free from Cl. ions. When 2.5 gm. of the dried silicic acid was left with 10 cc. *N* NH_4OH for 48 hours and then boiled exactly as in the other cases, only 0.1 m.e. of ammonia was taken up. Under similar experimental conditions, 2.5 gm. of aluminum oxide took up only 0.03 m.e. of ammonia.

TABLE 2

Ammonia retention by H-soils and pH values of the resulting ammonium soils

SOIL NO.	pH OF NH_4-SOIL	*T*/2	NH_3 TAKEN UP BY 100 GM. SOIL	SOIL NO.	pH OF NH_4-SOIL	*T*/2	NH_3 TAKEN UP BY 100 GM. SOIL
		m.e.	*m.e.*			*m.e.*	*m.e.*
M. 1	6.62	23.2	24.4	P.C. 5	7.85	10.6	7.4
2	6.93	12.0	11.6	8	7.72	19.6	16.6
3	6.53	14.6	13.4	9	7.57	7.7	6.7
4	6.79	12.8	11.4	10	7.59	19.8	22.4
5	6.57	20.0	18.6	11	6.77	26.0	22.7
6	6.72	10.0	9.2	14	7.41	24.0	16.4
7	6.93	28.0	23.6	15	7.18	5.7	5.0
8	5.96	28.2	26.2	17	7.68	6.5	6.9
9	5.96	12.4	8.8	20	7.58	3.8	4.0
10	6.25	16.6	20.4	21	7.62	11.4	12.3
11	6.24	14.0	13.0	25	7.64	3.7	1.0
12	6.92	16.2	19.8	27	7.40	50.4	49.8
13	6.55	9.4	9.6	29	7.60	43.0	48.3
14	6.73	10.6	10.6	31	7.78	12.9	16.3
15	6.06	13.5	14.6	39	7.24	9.6	7.5
16	6.40	5.8	6.2	40	7.50	9.8	8.4
17	7.12	10.0	13.6	43	7.34	10.2	10.4
18	6.80	15.6	17.0	44	7.86	6.4	5.6
21	6.19	14.2	22.6	45	7.60	5.2	4.5
22	6.51	12.3	12.0	48	7.98	5.4	5.7
23	6.83	12.5	14.4	49	7.78	14.1	16.4
P.C. 1	7.36	12.0	9.4	50	7.80	7.5	7.7
2	7.20	54.4	60.6	51	7.51	6.4	7.0
3	7.48	61.0	61.9				

The experiments, described in the foregoing, on ammonia retention by soil and buffer solutions were conducted at the boiling temperature. This was preferred on account of the ease of manipulation and the rapidity of reaction. The reaction if studied at the ordinary temperature does not show a definite end point within a reasonable time. Thus, when air was passed through a soil suspension neutralized with ammonia, equilibrium was not reached even after 48 hours. The apparatus consisted of a glass cylinder with a sintered glass bottom through which air could be forced, forming minute bubbles which

kept the soil suspension in the cylinder well stirred, and all the free ammonia was driven to a Reiset's apparatus containing standard acid. The ammonia driven out was determined by titrating the standard acid.

It was of interest to determine the final pH value of soils after the base-free soils were boiled with excess of ammonia. A number of soils, therefore, were acid treated and shaken with excess of ammonia for 48 hours. The excess of ammonia was then driven out by boiling, and the pH values were determined. For this purpose the boiled suspensions were cooled and diluted with water to make up for the loss of water on boiling. The results given in table 2 show that the ammonia retained by most of the soils is equivalent to $T/2$, which is determined by titration curves (4). This fact leaves no doubt that the soil acidoids react with ammonia exactly in the same way as do true acids.

SUMMARY

The reaction of ammonia with soils containing different amounts of exchangeable bases as well as with an ordinary buffer solution was studied.

The amount of ammonia reacting with the soils is a function of the pH value and represents the residual portion of the titration curves when the soil is partly neutralized with another base.

The amount of ammonia retained by base-free soil on boiling is equivalent to $T/2$ for that soil.

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THE DETERMINATION OF THE ORGANIC BASE-EXCHANGE CAPACITY OF SOILS[1]

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Since many of the important properties of soils are definitely related to their base-exchange nature, soil scientists have devoted considerable time to the study of base-exchange in an effort to obtain more information concerning the physical and chemical properties of soils.

Although it has been recognized that both the organic and the inorganic fractions of the soil play a rôle in the base-exchange processes, there has been a tendency to overemphasize the importance of the inorganic complex, as is shown by the detailed studies which have been devoted to it (16). On the other hand, fewer investigations have been concerned with the organic exchange complex. A number of investigators have demonstrated the absorbing property of humus, but, for the most part, their investigations have gone little beyond this point.

Observations made by various soil workers indicate that the organic fraction of soils, because of its very high base-exchange capacity, plays an extremely important part in base-exchange reactions (13, 27).

Van Bemmelen (26) pointed out that a sandy soil with sufficient organic matter might have as high an absorptive capacity as a clay soil.

Oden (23) found humic acid obtained from the organic matter of peats to have a base-exchange capacity of 294 m.e.[3]

In some of his earlier work, Gedroiz (9) expressed the opinion that the base-exchange capacity of humates was "probably inconsiderable in comparison with that of the mineral part of the soil," but later (10) he suggested that the humus portion of the soil may have a greater effect than the mineral portion on the base-exchange capacity.

Hissink (12, 14) advanced the view that the humate portion of the soil has a far greater absorptive power than has the clay portion.

Baver (6) found that the absorptive capacity in certain soils due to organic matter varied from 30 to 60 per cent of the total capacity, which indicates that

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[3] m.e. is used throughout this paper to indicate milligram equivalents per 100 gm.

the absorptive capacity of surface soils is largely controlled by the organic matter present.

McGeorge (20) showed that the "ligno-humate" extract from soils had a maximum base-exchange capacity of 431 m.e.

Mitchell (22) found 41 to 65 per cent of the base-exchange capacity in a variety of mineral soils to be due to organic matter.

In consideration of the foregoing observations and of others of a similar nature, which indicate the important part played by organic matter in base-exchange processes, it seems that investigations dealing with organic base-exchange materials in soils would be profitable. Possibly a reason for not making an extensive study of the organic base-exchange capacities of soils is the lack of a definite method for making such determinations. The relatively few determinations of the organic base-exchange capacity of soils have been made in one of the following three ways:

(*a*) The organic portion of the soil is removed by an alkaline extracting solution and reprecipitated by the addition of a mineral acid, and the determination of the base-exchange capacity of the organic matter is made directly. [See Arnold and Page (3), McGeorge (20), and Oden (23).]

(*b*) The organic matter of the soil is destroyed by gentle ignition, and the base-exchange capacity is determined before and after treatment, the difference representing the exchange capacity of the organic matter. [See Mitchell (22), Kerr (17), McGeorge (21).]

(*c*) The organic matter of the soil is destroyed by treatment with hydrogen peroxide, and the base-exchange capacity is determined indirectly as in (*b*). [See Robinson (25), McGeorge (19), Baver (6), Gedroiz (9).]

In organic base-exchange determinations carried out in accordance with any of the three foregoing procedures, serious objections may arise.

The chief objection to (*a*) is that in extracting the organic matter with an alkaline solution and subsequently determining the exchange capacity of the extracted organic matter, it has been found (20) that the exchange capacity of the organic matter depends upon the method used in preparing or separating it from the mother substance. A second objection is that this procedure requires considerable time.

In (*b*) there is a rather serious threat of dehydrating the soil minerals by the ignition and thus reducing the base-exchange capacity of the inorganic complex. Kelley, Dore, and Brown (16) show that heating bentonites, soil colloids which are free from organic matter, and zeolites above 350°C. significantly alters their base-exchange capacity; and that the base-exchange capacity of zeolites is affected at a much lower temperature. A second serious objection to this procedure is that 7 or 8 hours of ignition are required to bring about the destruction of the organic matter.

In (*c*) there is a possibility that the peroxide treatment might affect the inorganic base-exchange capacity in a manner similar to that of ignition. McGeorge (19) showed that digestion with H_2O_2 does not affect the exchange capacity of synthetic zeolites. He concluded that there was considerable

evidence in favor of using H_2O_2 as a reagent in the "difference method" for determining the exchange capacity of organic matter. The principal objection to the use of this procedure, in previous work, for determining the organic base-exchange capacity is the inconsistency in the manner in which the oxidations have been carried out and the lack of definite information concerning the effects of various concentrations of peroxide in making such determinations. Some of the variations in the manner of H_2O_2 treatment in organic base-exchange studies are indicated in the following brief review of the literature:

TABLE 1

Description of soils used

SAMPLE NUMBER	FIELD	PLOT	TREATMENT*	ORGANIC CARBON	SOIL TYPE†
				per cent	
S-6754	Ewing	109	RLPK	1.27	No. 3, Hoyleton silt loam
S-6755	Ewing	110	0	1.14	No. 3, Hoyleton silt loam
S-6756	Hartsburg	409	RLPK	4.72	Youthful Grundy clay loam
S-6757	Hartsburg	410	0	3.54	Youthful Grundy clay loam
S-6759	Minonk	409	RLPK	4.77	No. 152, Drummer clay loam
S-6758	Minonk	410	0	4.54	No. 152, Drummer clay loam
S-6760	Carthage	309	RLPK	2.67	Edina silty clay loam
S-6761	Carthage	310	0	2.15	Edina silty clay loam
S-6762	West Salem	409	RLPK	1.31	Cisne silt loam, with some slick spots
S-6763	West Salem	501	0	1.00	Cisne silt loam, with some slick spots
S-6764	Toledo	109	RLPK	1.46	Cisne silt loam
S-6765	Toledo	110	0		Cisne silt loam
S-6766	Clayton	409	RLPK	2.15	Edina silt loam
S-6767	Clayton	410	0	1.93	Edina silt loam
S-6768	Elizabethtown	109	RLPK	0.81	Clement silt loam
S-6769	Elizabethtown	105	0	0.39	Clement silt loam. Serious erosion—more than that on plot 109
Peat	Manito, Mason County, Illinois				Deep peat

* R = Crop residues; L = Limestone; P = Rock phosphate; K = Kainite.

† Personal communication from R. S. Smith, 1937.

Gedroiz (11) treated a 25-gm. sample of soil with a 10 per cent solution of H_2O_2, warmed to 30 or 40°, and dried the treated soil in the air before determining the residual base-exchange capacity. Powers (24) used 30 per cent H_2O_2 to remove the organic portion of the ligneous fraction of a peat soil and found that it destroyed most of the base-exchange capacity. Kerr (18) used both 3 and 6 per cent H_2O_2 at a temperature of about 60°C. McGeorge (19) treated a 1-gm. sample of soil with 30 cc. of 15 per cent H_2O_2 until no further evolution of bubbles occurred. Mitchell (22) used both 3 and 15 per cent peroxide with repeated treatments.

None of the aforementioned workers,[4] however, have described a method which could be accepted as a standard for the determination of the organic base-exchange capacity of soils, since they did not show that the method used was efficient in giving the *maximum* decomposition of the organic exchange material nor did they show the specific conditions necessary for an effective peroxide decomposition.

It is the purpose of this paper to describe a method for the determination of organic base-exchange capacity in soils, indicating what treatment brings about the maximum reduction of the total exchange capacity; to show the effect on the exchange capacity of varying concentrations and volumes of H_2O_2 as well as the effect of the number of treatments; and to demonstrate the effect of manganese compounds, carbonates, and changes in H-ion concentration on the efficiency of the H_2O_2 treatment.

The soils used in this study (table 1) were fertilized and unfertilized soils (5) obtained from the Illinois Agricultural Experiment fields, representing varying content of organic matter.

METHOD OF INVESTIGATION

The method of investigation described below is also the one recommended as a routine procedure for organic base-exchange capacity, except that a definite quantity of H_2O_2 (40 cc. of 15.0 per cent) is used in routine work on soils similar to the ones included in this investigation.

Hydrogen peroxide treatment

Five-gram samples of soil were weighed in duplicate and placed in 400-cc. pyrex beakers, H_2O_2 solution was added, and the beakers were covered with watch glasses and placed on a steam bath. The soil was digested for approximately 1 hour, the watch glasses were then removed, and the liquid was completely evaporated. Where two treatments with peroxide were made, the second, which was the same as the first, was made after complete evaporation of the liquid of the first treatment. After the second treatment, the liquid was again completely evaporated, and the base-exchange capacity was determined by a modification of the method recommended by Chapman and Kelley (8). Mitchell (22) objected to the use of H_2O_2 in determining the organic base-exchange capacity of soils on the grounds that peroxide-treated soils are difficult to leach. Evaporation to dryness, as in the foregoing procedure, eliminates this difficulty.

[4] After this work was completed, Bartlett, Ruble, and Thomas (4) reported a study to estimate the amount of organic matter in the exchange complex of a number of Maryland soils. Their treatment with H_2O_2 to destroy the organic base-exchange complex apparently was not sufficient, and an end point in the destruction of the organic exchange capacity was evidently not reached in the majority of the cases given. Their values for the percentage of organic matter oxidized range from 14.3 to 97.5, and our work shows that over 80 to 90 per cent of the organic matter must be decomposed in order to destroy the measurable organic base-exchange capacity.

Base-exchange capacity

Five- or ten-gram samples of soil (weighed in duplicate) were placed in 250-cc. beakers, and 50 cc. of normal, neutral NH_4Ac was added.[5] The solution in the beakers was stirred occasionally for a half hour, and then poured into 9-cm. Buchner funnels, where it was leached with more NH_4Ac by suction into 1-liter Erlenmeyers until the total volume of leachate was 500 cc. The excess NH_4Ac was washed from the soil with 300 cc. of absolute methanol, which had been brought to neutrality with NH_4OH.

Following the washing by methanol, the soils in the Buchners were leached with 300 cc. 0.1 *N* HCl, the leachate having been received in clean Erlenmeyers. The leachate was transferred quantitatively into 800-cc. pyrex Kjeldahl flasks, about 5 gm. of NaOH pellets were added to each flask, and the ammonia was distilled into 500-cc. Erlenmeyers containing 50 cc. of 0.1 *N* HCl. The excess HCl was back-titrated with 0.1 *N* NaOH, using a mixed indicator (methyl red and methylene blue) which is colorless at the end point. When a 5-gm. sample is used, the base-exchange capacity is twice the number of cubic centimeters of 0.1 *N* acid used.

Since in the foregoing procedure for inorganic base-exchange capacity the soils are subjected to drying on the steam bath, a treatment not included in the regular base-exchange capacity method, the possible effect of this treatment on the total base-exchange capacity of determination was studied. Soils varying in their total capacity were given this steam bath treatment, the H_2O_2 being omitted, and then their base-exchange capacity was determined. No effect of the steam bath treatment could be observed in the results obtained.

DISCUSSION OF RESULTS

Five soils, having various organic matter contents, and one peat were given a single treatment with 40 cc. of H_2O_2 of concentrations ranging from 3.75 to 30.0 per cent. Table 2 shows the effects of these treatments on the base-exchange capacities. A single treatment with 15 to 22.5 per cent peroxide solution brought about a rather consistent maximum reduction of the base-exchange capacity in the five soils, but with the peat 22.5 per cent H_2O_2 was needed to give the maximum reduction in exchange capacity.

In order to determine whether peroxide less concentrated than 15 per cent would bring about the maximum reduction in the exchange capacity as effectively as the stronger solution, a detailed study was made in which a greater number of soils was treated with both 11.25 and 15.0 per cent peroxide. Although the results in table 2 indicated the possibility of using a weaker solution of peroxide, the results of the more detailed study (table 3) show that the 15.0 per cent peroxide is somewhat more effective than the 11.25 per cent in producing the maximum reduction of the base-exchange capacity. The reproducibility of the determination for the peroxide-treated samples was

[5] The H_2O_2-treated soils are treated with NH_4Ac in the beaker in which the H_2O_2 digestion has been made.

studied, and it was found that repeated determinations checked within 1 m.e. The organic base-exchange capacity, as calculated by subtracting the value of the base-exchange capacity after treatment with 15.0 per cent peroxide from

TABLE 2

Base-exchange capacity before and after treatment with H_2O_2 of varying concentration

SAMPLE NUMBER	ORIGINAL BASE-EXCHANGE CAPACITY	BASE-EXCHANGE CAPACITY AFTER TREATMENT WITH 40 CC. H_2O_2					
		3.75 per cent	7.5 per cent	11.25 per cent	15.0 per cent	22.5 per cent	30.0 per cent
	m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
S-6756	37.6	25.2	24.7	25.9	24.2	23.8	24.8
S-6759	37.1	21.8	20.8	20.8	21.0	21.7	20.6
S-6761	24.3	16.2	17.0	17.2	16.5	16.4	17.0
S-6764	11.4	8.8	9.8	9.3	8.6	8.2	8.8
S-6767	19.9	13.6	13.2	13.6	12.2	12.4	12.6
Peat	105.8	69.6	28.6	19.2	16.2	10.8	10.2

TABLE 3

Comparative effects of 11.25 and 15.0 per cent H_2O_2 on the removal of the organic base-exchange material

SAMPLE NUMBER	ORIGINAL BASE-EXCHANGE CAPACITY (*a*)	BASE-EXCHANGE CAPACITY AFTER TREATMENT WITH 40 CC. H_2O_2			
		11.25 per cent (*b*)	15.0 per cent (*c*)	Organic base-exchange capacity	
				a–c	Per cent of total base-exchange capacity
	m.e.	*m.e.*	*m.e.*	*m.e*	
S-6758	37.9	22.4	21.6	16.3	43.0
S-6759	37.1	20.8	21.0	16.1	43.4
S-6756	37.8	26.5	24.0	13.8	36.6
S-6757	37.6	25.9	24.2	13.4	35.6
S-6760	26.0	18.7	16.6	9.4	36.1
S-6766	20.9	12.7	12.2	8.7	41.6
S-6761	24.3	17.2	16.5	7.8	32.0
S-6767	19.9	13.6	12.2	7.7	38.7
S-6754	11.3	7.3	7.3	4.0	35.4
S-6764	11.4	9.3	8.6	2.8	24.6
S-6763	9.9	7.8	7.2	2.7	27.3
S-6762	10.0	8.1	7.6	2.4	24.0
S-6769	16.1	15.1	14.6	1.5	9.3
S-6768	10.2	9.6	9.2	1.0	9.8
S-6755	8.8	8.1	8.2	0.6	6.8
Peat	105.8	19.2	16.2	...	...

the base-exchange capacity of the original soil without treatment varied from 0.6 to 16.3 m.e. in the soils studied, and constituted from 6.8 to 43.4 per cent of their total base-exchange capacity.

In addition to the effect of variation in the concentration of H_2O_2 on the reduction of base-exchange capacity, the effects produced by variation in volume of H_2O_2 used and the number of treatments given were also studied. Table 4 shows the data obtained. When the volume of peroxide solution used increased to 80 cc. and one treatment was given, the additional volume had no significant effect on the reduction of the base-exchange capacity. Two treatments of 40 cc. each of 15.0 per cent peroxide produced a reduction of the base-exchange capacity that was not significantly different from that produced by only one treatment of the same kind. Four treatments of 40 cc. each of 30.0 per cent peroxide were not so effective as a single treatment of the same kind in reducing the base-exchange capacity. There was an indication that the repeated treatments with the more concentrated peroxide had the effect of increasing the base-exchange capacity of soils S-6764 and S-6767.

Although an increased volume of peroxide had no effect in further reducing the base-exchange capacity of soils that were not unusually high in organic

TABLE 4

Base-exchange capacity of soils remaining after different treatments

SAMPLE NUMBER	NO TREATMENT	40 cc. H_2O_2				80 cc. H_2O_2 7.5 PER CENT 1 TREATMENT
		15.0 per cent 1 treatment	15.0 per cent 2 treatments	30.0 per cent 4 treatments	7.5 per cent 1 treatment	
	m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
S-6757	37.6	24.2	23.4	24.0	24.7	24.9
S-6759	37.1	21.0	21.6	21.1	20.8	20.9
S-6761	24.3	16.5	17.6	17.2	17.0	17.7
S-6764	11.4	8.6	9.8	10.2	9.8	9.9
S-6767	19.9	12.2	13.2	14.0	13.2	13.4

matter, it was thought advisable to observe the effect of increased volume of peroxide on the reduction of the base-exchange capacity of the peat sample. Both 15.0 and 22.5 per cent peroxide were used, and the volume was increased to 60 and to 80 cc. The following results indicate that the end point in the reduction of the base-exchange capacity had not been reached with the treatment using 40 cc. of 15.0 per cent peroxide:

VOLUME H_2O_2	BASE-EXCHANGE CAPACITY AFTER TREATMENT	
	15 per cent H_2O_2	22.5 per cent H_2O_2
cc.	*m.e.*	*m.e.*
40	16.2	10.8
60	10.9	10.8
80	9.6	11.4

With increased volume or strength of peroxide, the values for the base-exchange capacity of the oxidized material ranged from 9.6 to 11.4 m.e. Since variations within this range may be considered to be within experimental

error, in view of the high base-exchange capacity of the original sample of peat, it is quite certain that these values represent an end point in the oxidation.

TABLE 5

Organic carbon as determined by the chromic acid reduction method

SAMPLE NUMBER	CARBON		PER CENT OF TOTAL ORGANIC CARBON REMOVED
	Original	Treated	
	per cent	*per cent*	
S-6754	1.27	0.15	88
S-6755	1.14	0.12	89
S-6756	4.72	0.27	94
S-6757	3.54	0.05	99
S-6758	4.54	0.23	95
S-6759	4.77	0.42	91
S-6760	2.67	0.39	85
S-6761	2.15	0.19	91
S-6762	1.31	0.21	84
S-6763	1.00	0.15	85
S-6764	1.46	0.11	92
S-6766	2.15	0.18	92
S-6767	1.93	0.16	92
S-6768	0.81	0.10	88
S-6769	0.39	0.08	79

TABLE 6

Base-exchange capacity of the organic matter oxidized by treatment with 40 cc. of 15.0 per cent H_2O_2

SAMPLE NUMBER	ORGANIC BASE-EXCHANGE CAPACITY	ORGANIC MATTER OXIDIZED	BASE-EXCHANGE CAPACITY OF OXIDIZED ORGANIC MATTER
	m.e.	*per cent*	*m.e.**
S-6758	16.3	7.41	220
S-6759	16.1	7.49	215
S-6756	13.8	7.66	180
S-6757	13.4	6.02	223
S-6760	9.4	4.10	229
S-6766	8.7	3.39	257
S-6761	7.8	3.38	230
S-6767	7.7	3.05	252
S-6754	4.0	1.93	207
S-6764	2.8	2.32	120
S-6763	2.7	1.46	185
S-6762	2.4	1.89	127
S-6769	1.5	0.53	283
S-6768	1.0	1.22	82
S-6755	0.6	1.76	34

*Per 100 gm. of organic matter.

Total carbon was determined by the chromic acid reduction method (2) on the soils used, both before and after oxidation with 15.0 per cent peroxide

(table 5). The percentage of carbon removed varied from 79.0 to 99.0, the average being slightly over 90.0 per cent.

In table 6 the base-exchange capacity of the organic matter removed was calculated and found to vary from 34 to 283 m.e. The values for the base-exchange capacity of the organic matter itself are of significance, however, only in the soils which have a sufficiently high organic base-exchange capacity to make the calculation valid. For instance, an experimental variation of 0.5 m.e. in the determination of the exchange capacity of soil S-6755, both before and after oxidation, the variations falling in opposite directions, would increase the value for the organic base-exchange capacity by 1 m.e., giving an organic base-exchange value of 1.6 m.e. This would produce an apparent increase of 267 per cent in the base-exchange capacity of the organic matter, due entirely to normal experimental error.

It was observed that the amount of the R_2O_3 precipitate in the NH_4Ac-HCl filtrate of the peroxide-treated soil was much greater than that in the filtrate of the untreated soil. Gedroiz (9) found that the amount of H_2O-soluble mineral substances increased six times in peroxide-treated soil as compared to the untreated soil.

Relationship of easily and difficultly oxidized organic matter of soils to their organic base-exchange capacity

It might be supposed that the H_2O_2 treatment of soils would result in the initial removal of the most decomposed portion of organic matter, a more resistant portion being left for later oxidation. A study was made, therefore, to determine whether the base-exchange capacity was more closely associated with the portion first oxidized or with that which was oxidized later. This was determined by treating two different soils with varying quantities of H_2O_2 and determining their base-exchange capacity and organic matter after each treatment. These results (table 7) show that the organic matter decomposed in the latter part of the treatments has a higher base-exchange capacity than that of the organic matter initially decomposed, and they suggest that the small percentage of organic carbon which still remains in most soils after the H_2O_2 treatment might have a significant base-exchange capacity. An additional study was made to find whether this were the case.

Two soils in which the organic residue (remaining after one treatment) was rather large were subjected to more severe H_2O_2 treatment, and the percentage of organic carbon remaining was brought down to a much lower value.

It was found that the removal of much of this remaining organic matter in these soils did not affect their base-exchange capacity. In soil S-6759 (table 8) reduction of the organic carbon from 0.42 to 0.16 per cent, a removal of more than 50 per cent of the organic carbon which remained, should have resulted in a decrease of about 1 m.e. in base-exchange capacity, provided this last-oxidized material had a base-exchange capacity comparable to that of the first-oxidized organic matter, as shown in table 7. However, no decrease in base-exchange capacity occurred.

The conclusion to be drawn from these results is that the portion of organic matter most easily oxidized, such as that oxidized by dilute concentrations of H_2O_2, has less base-exchange capacity than that which is oxidized only with

TABLE 7

Base-exchange capacity of the organic matter oxidized by varying concentrations of H_2O_2

TREATMENT	TOTAL BASE-EXCHANGE CAPACITY AFTER TREATMENT	ORGANIC BASE-EXCHANGE CAPACITY AFTER TREATMENT	ORGANIC CARBON AFTER TREATMENT	SUCCESSIVE DECREASES		BASE-EXCHANGE CAPACITY OF OXIDIZED ORGANIC MATTER
				Base-exchange capacity	Organic carbon	
	m.e.	*m.e.*	*per cent*	*m.e.*	*per cent*	*m.e.*
Soil S-6757						
0	37.6	13.4	3.54	...	....	.....
40 cc. 0.95 per cent H_2O_2	33.3	9.1	1.77	4.3	1.77	141.0
40 cc. 1.9 per cent H_2O_2	28.9	4.7	0.81	4.4	0.96	267.0
40 cc. 15.0 per cent H_2O_2	24.2	0	0.05	4.7	0.76	359.0
Soil S-6766						
0	20.9	8.7	2.15	...	....	.....
40 cc. 0.95 per cent H_2O_2	18.4	6.2	1.17	2.5	0.98	148.0
40 cc. 1.9 per cent H_2O_2	15.1	2.9	0.70	3.3	0.47	408.0
40 cc. 15.0 per cent H_2O_2	12.2	0	0.18	2.9	0.52	325.0

TABLE 8

Effect of further removal of organic carbon by successive H_2O_2 treatments on the base-exchange capacity*

SOIL	BASE-EXCHANGE CAPACITY	ORGANIC CARBON	DECREASES		BASE-EXCHANGE CAPACITY OF ORGANIC MATTER REMOVED
			Base-exchange capacity	Organic carbon	
	m.e.	*per cent*	*m.e.*	*per cent*	*m.e.*
S-6762					
Original	10.0	1.31	...	....	...
After 1 treatment	7.6	0.21	2.4	1.10	126
After 2 treatments	7.7	0.07	0	0.14	0
S-6759					
Original	37.1	4.77	....	....	...
After 1 treatment	21.0	0.42	16.1	4.35	215
After 2 treatments	21.6	0.29	0	0.13	0
After 3 treatments	21.1	0.16	0	0.13	0

* Each treatment consisted of 40 cc. of 15 per cent H_2O_2.

more concentrated H_2O_2 (table 7). The residue from the single treatment with 15.0 per cent H_2O_2, however, does not possess any significant base-exchange capacity as is shown by the effect of the additional treatments (table 8).

Effect of acidity and manganese compounds on destruction of organic base-exchange capacity by peroxide

A study was made to determine the effect of H-ion concentration on the destruction of the organic base-exchange complex with peroxide in the presence of manganese compounds.

Variations in H-ion concentration affect the decomposition of the organic matter in a soil to a marked degree. Hosking (15) showed that the decomposition of soil organic matter by H_2O_2 is a function of the hydrogen-ion concentration and that the greatest degree of oxidation takes place in an acid medium.

The soils used in this study have pH values ranging from 4.95 to 7.40 and contain no carbonates. The soils of a low base-exchange capacity contain concretions high in iron and manganese chiefly as MnO_2. The replaceable manganese in certain of these soils runs as high as 150 an acre.

Best (7) worked on the reaction between MnO_2 and H_2O_2 with variation in the H-ion concentration of the medium in which the reaction takes place. In alkaline solution, a catalytic decomposition of H_2O_2 takes place with $Mn(OH)_2$ as the probable intermediate compound:

$$MnO_2 + H_2O_2 = Mn(OH)_2 + O_2$$

$$Mn(OH)_2 + H_2O_2 = MnO_2 \cdot H_2O + H_2O$$

In acid solution, the following reaction, which is stoichiometric, takes place:

$$MnO_2 + H_2O_2 + H_2SO_4 = MnSO_4 + 2H_2O + O_2$$

In consideration of the above reactions, the presence of a small quantity of MnO_2 in alkaline soils would have a considerable retarding effect on the destruction of the organic base-exchange capacity of these soils with peroxide. If the H-ion concentration of the soils is on the acid side, a greater amount of MnO_2 would be required to decompose the peroxide and seriously interfere with the oxidation.

A number of different substances, including manganese compounds and alkaline and acid reagents, were added to samples of soil S-6759, and the base-exchange capacity was determined after a single treatment with 40 cc. of 15.0 per cent peroxide. The results in table 9 show that the addition of certain substances has a pronounced effect on the destruction of the organic base-exchange capacity by peroxide.

The results show that the addition of 100 mgm. of MnO_2 brought about the decomposition of the H_2O_2 before any of the organic exchange capacity was destroyed. The addition of the small amount of acid or base seemed to have no effect on the decomposition of the H_2O_2 by this large quantity of MnO_2.

Manganese sulfate was added to determine whether it would produce the same effect on the H_2O_2 as did the MnO_2, since in alkaline solution, $MnSO_4$ is oxidized to MnO_2 (or an oxide closely related to MnO_2), as follows:

$$Mn^{++} + H_2O_2 + 2OH^- = MnO_2 + 2H_2O$$

The addition of $MnSO_4$ affects the peroxide oxidation only on the basic side. This was the result expected in consideration of the foregoing reaction. Where 1 gm. of $CaCO_3$ was added, a serious interference with the destruction of the organic base-exchange capacity was noted, although the oxidizing action of the H_2O_2 was not altogether prevented. Additional treatments with peroxide probably would have effected the complete destruction of the organic base-exchange capacity.

In all the cases observed, the MnO_2 rendered the H_2O_2 ineffective. Where the MnO_2 is present in small quantities in the soils studied and where the H-ion concentration is on the acid side, the interference is not great. Alexander and Byers (1) indicated that the interfering effect of MnO_2 would be greatly reduced by the addition of acid to the sample undergoing oxidation. Additional H_2O_2 treatments for soils containing large amounts of manganese would, however, be advisable.

TABLE 9

Effects of various substances on the effectiveness of H_2O_2 treatment

Single treatment with 40 cc. of 15 per cent H_2O_2; 5 gm. S-6759

SUBSTANCE ADDED	BASE-EXCHANGE CAPACITY
	m.e.
None	21.0
100 mgm. MnO_2	38.3
100 mgm. MnO_2 + 0.15 cc. conc. NH_4OH	37.6
100 mgm. MnO_2 + 0.15 cc. conc. HAc	37.8
100 mgm. $MnSO_4$	20.9
100 mgm. $MnSO_4$ + 0.15 cc. conc. NH_4OH	36.6
100 mgm. $MnSO_4$ + 0.15 cc. conc. HAc	21.2
1 gm. $CaCO_3$	27.0
Original base-exchange capacity	38.4

The foregoing results suggest the addition of a few drops of concentrated $(NH_4)OH$ to a H_2O_2-soil mixture as a means of quickly and completely removing the H_2O_2 remaining after the oxidation. This was verified by adding a few drops of ammonia to several different H_2O_2-soil mixtures. Within less than a minute no test for peroxide could be obtained.

Since, in the absence of MnO_2 or other interfering substances, hydrogen peroxide is a more powerful oxidizer in alkaline solution than in acid, the effect of 25 cc. of 15.0 per cent H_2O_2, under acid and alkaline conditions, on a 1-gm. sample of filter paper was determined. When 0.15 cc. conc. NH_4OH was added to the H_2O_2, 9.78 per cent of the filter paper was decomposed, compared to a 6.32 per cent decomposition which occurred when 0.15 cc. conc. HAc was added to the H_2O_2. Thus, it is probable that if soils were entirely free from manganese, oxidation with H_2O_2 could be carried out to advantage in alkaline solution, but since manganese-free soils are rare, an acid medium is desirable for most oxidation work.

SUMMARY AND CONCLUSIONS

In the Illinois soils studied, the organic matter ranged from approximately 13,500 to 165,000 pounds an acre, and the organic base-exchange capacity varied from 0.6 to 16.3 m.e., constituting from 6.8 to 43.4 per cent of the total base-exchange capacity of the soils. The end point in the destruction of the organic base-exchange capacity was reached by using a single 40-cc. treatment with 15.0 per cent H_2O_2. In the sample of peat studied, one treatment with 40 cc. of 22.5 per cent H_2O_2 was necessary to reach the end point in the destruction of the organic base-exchange capacity.

Manganese dioxide in acid, neutral, and alkaline media; manganous salts in alkaline media; and $CaCO_3$ interfere considerably with the destruction of the organic base-exchange capacity of soils by peroxide oxidation. The destruction of the organic base-exchange capacity of soils in which these substances are present in unusually large amounts presents a special problem which has not yet been satisfactorily worked out. Such soils, as well as soils very high in organic matter, may need more severe treatment than that found satisfactory for the Illinois soils studied. In applying to other soils the method described, it is essential, therefore, to make some study of the amount of peroxide necessary for maximum decomposition.

In general, the progress of the decomposition of the organic matter can be observed in dark-colored soils by the change in color. Soils giving a violent initial reaction with no corresponding significant reduction in base-exchange capacity are usually high in active manganese or carbonates. In such soils, additional treatments with H_2O_2 (40 cc. of 15 per cent, for example) to which a few drops of conc. HAc has been added, will probably be needed to effect a complete decomposition of the organic base-exchange material.

It is recommended that, in studying soils similar in nature to the soils included in the present work (the peat excepted), one treatment with 40 cc. of 15 per cent peroxide be used in the method described. This recommendation is based on the fact that although weaker concentrations of H_2O_2 gave fair results, more peroxide should probably be used in routine work as a matter of precaution. This amount should be sufficient to take care of the likely variations in the upland prairie and timber types.

With unknown soils, two or more concentrations or amounts of peroxide should be used in preliminary studies to determine the amount needed for decomposition.

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